CANADIAN JOURNAL OF RESEARCH

VOLUME 20 AUGUST, 1942 NUMBER 8

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NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

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Subscription rates, postage paid to any part of the world (effective 1 April, 1939), are as follows:

	Annual	*Single Copy*
A and B	$ 2.50	$ 0.50
C and D	2.50	0.50
Four sections, complete	4.00	—

The Canadian Journal of Research is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. All correspondence should be addressed:

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. C. AUGUST, 1942 NUMBER 8

RESEARCHES ON DROUGHT RESISTANCE IN SPRING WHEAT

II. THE EFFECT OF TIME OF DAY ON SURVIVAL OF PLANTS DURING EXPOSURE TO ARTIFICIAL DROUGHT[1]

BY C. B. KENWAY[2], H. B. PETO[3], AND K. W. NEATBY[4]

Abstract

The time of day during which tests are conducted has a pronounced effect on the damage sustained by wheat plants exposed to artificial drought. When the day is divided into four six-hour periods, beginning at 6.00 a.m., the period from 6.00 a.m. to 12.00 noon provides the most severe test. In 12-hr. and 18-hr. exposures, relatively severe damage to the plants is associated with the inclusion of the 6.00 a.m. to 12.00 noon period.

Introduction

During the past five years, numerous tests of wheat varieties and strains have been conducted in the artificial drought machine described by Kenway and Peto (1). The results of preliminary trials revealed relatively enormous differences between the survival of experimental plants from one replicate to another. Since the machine had a capacity of only 25 6-in. pots, these differences had an important bearing on the design of experiments involving large numbers of varieties and strains. It was found that the time of day during which plants were exposed to artificial drought had a direct bearing upon the result. The purpose of this paper is to summarize data that serve to illustrate the relation between survival in the artificial drought machine and the time of day during which tests are conducted.

Peto (3) concluded, from experiments conducted in 1936, that wheat plants suffered relatively severe injury when the period extending from 6.00 a.m. to 12.00 noon was included in the test. He found that a 12-hr. exposure, beginning at 12.00 noon, effected approximately the same damage as a six-hour exposure beginning at 6.00 a.m. Laude (2) has obtained similar results with wheat, barley, corn, and sorghum. He states that ". . . . . the daily

[1] *Manuscript received May 20, 1942.*

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maximum resistance to heat was attained by plants at about midday and continued during the afternoon. The minimum resistance prevailed early in the morning."

Materials and Methods

The material consisted of a number of varieties and strains of *Triticum vulgare* and two varieties of *T. durum*. In Experiment 1, there were 22 varieties, in Experiments 2 and 5 there were 25, while Experiments 3, 4, and 6 included 75.

The plants were subjected to treatment about a week before heading, though different varieties were, of necessity, at different stages of development. They were grown in ordinary 6 in. gardener's pots with drainage holes in the bottoms. The pots were watered before and after treatment by placing them in shallow trays which were filled with water. Since the porosity and, hence, evaporation rate varied from one pot to another, the pots were painted on the outside with asphaltum. In the early experiments, five plants were grown in each pot; but this number was reduced to four in later experiments.

The temperature in the machine varied not more than one degree above or below 110° F. The relative humidity varied from 14 to 23% from one experiment to another, but not more than 3% within six-hour tests, 5% within 12-hr. tests, and 7% within 18-hr. tests. The velocity of the air current was maintained at approximately 10 miles per hour.

The survival indices were based on the estimated percentage of uninjured tissue per pot. At first an attempt was made to classify each plant; but, finally, it was concluded that more consistent results could be secured by placing the pots on a bench in order of the severity of damage, and assigning a percentage survival figure to each.

Experimental Results

The results are assembled in Table I. The four periods of the day are designated as follows:

A — 6 a.m. to 12 noon.
B — 12 noon to 6 p.m.
C — 6 p.m. to 12 midnight.
D — 12 midnight to 6 a.m.

Each entry in the table, except the mean totals, represents the total survival percentages of all varieties in the test concerned. Thus in the six-hour exposures of Experiment 1 (22 varieties), the average percentage survival in the *A* period is 368/22, or 16.7%. In the *D* period it is 958/22, or 43.5%. In experiments involving 25, or 22, varieties, each figure is the result of a single "run", the capacity of the machine being 25 pots. Experiments 3, 4, and 6 each included 75 varieties and, consequently, three runs are involved in each entry.

In designing Experiment 1, no provision was made for separating the variance due to periods from that due to replicates, the reason being that the

TABLE I

TOTAL SURVIVAL VALUES AND MEAN TOTALS OF ALL VARIETIES TESTED IN DIFFERENT PERIODS OF THE DAY

	Six-hour exposures			
	A 6 a.m. – 12 noon	*B* 12 noon – 6 p.m.	*C* 6 p.m. – 12 midnight	*D* 12 midnight – 6 a.m.
Experiment 1. 22 varieties; August 4 and 5, 1936	368 —	712 642	801 572	958 —
Mean totals	**368**	**677**	**686**	**958**
Experiment 2. 25 varieties; November 3 to 12, 1936	900 458 959	1625 — —	1757 — —	1166 — —
Mean totals	**772**	**1625**	**1757**	**1166**
Experiment 3. 75 varieties; February 16 to 21, 1937	1692 1123	1828 2918	1609 2272	— —
Mean totals	**1408**	**2373**	**1940**	—
Experiment 4. 75 varieties; May 9 to 14, 1937	3980 4750	5480 6000	5070 5620	— —
Mean totals	**4365**	**5740**	**5345**	—
	12-hr. exposures			
	AB 6 a.m. – 6 p.m.	*BC* 12 noon – 12 midnight	*CD* 6 p.m. – 6 a.m.	*DA* 12 midnight – 12 noon
Experiment 1. 22 varieties; August 5 to 8, 1936	— — —	325 308 317	— — —	75 112 104
Mean totals	—	**317**	—	**97**
Experiment 2. 25 varieties; November 3 to 12, 1936	122 —	756 883	156 —	343 526
Mean totals	**122**	**820**	**156**	**434**
Experiment 5. 25 varieties; May 17 to 22, 1937	1300 1530	1380 1650	1270 1770	940 1360
Mean totals	**1415**	**1515**	**1520**	**1150**

TABLE I—*Concluded*

TOTAL SURVIVAL VALUES AND MEAN TOTALS OF ALL VARIETIES TESTED IN DIFFERENT PERIODS OF THE DAY—*Concluded*

	18-hr. exposures			
	ABC 6 a.m. – 12 midnight	*BCD* 12 noon – 6 a.m.	*CDA* 6 p.m. – 12 noon	*DAB* 12 midnight – 6 p.m.
Experiment 1. 22 varieties; August 8 to 13, 1936	0 —	312 —	319 195	0 0
Mean totals	**0**	**312**	**257**	**0**
Experiment 2. 25 varieties; November 3 to 12, 1936	116 — —	390 250 512	269 — —	251 — —
Mean totals	**116**	**384**	**269**	**251**

	Four-hour exposures		
	$\frac{2}{3}A$ 6 a.m. – 10 a.m.	$\frac{1}{3}A+\frac{1}{3}B$ 10 a.m. – 2 p.m.	$\frac{2}{3}B$ 2 p.m. – 6 p.m.
Experiment 6. 75 varieties; November 25 to December 1, 1937	1860 1260	3190 2950	3690 3780
Mean totals	**1560**	**3070**	**3735**

chief purpose was to classify varieties and strains on the basis of resistance to treatment, and the differential effect of periods on survival was not fully appreciated until this experiment was completed. The data derived from 12-hr. exposures, however, were adequate for statistical analysis, since there were three replicates in each of the *BC* and *DA* periods, and none in the other two. It is apparent (Table II) that the difference between the mean survivals of the *BC* (12 noon to 12 midnight) and *DA* (12 midnight to 12 noon) periods is highly significant.

Experiment 2, involving 6-, 12- and 18-hr. exposures, was designed so that the *A* period would be included in one-half of the replicates and not included in the other half. In the analyses (Table II), therefore, one degree of freedom was involved in the variance for exposure periods. In the six-hour exposures, the *A* period was much more damaging to the plants than were the others. The results of the 12-hr. exposures are not fully in accord with expectation (note Experiment 5), since the *CD* period was more damaging than *DA*. The results of the 18-hr. exposures suggest that the *A* period is more damaging when it occurs at the beginning of a run (*ABC*) than when it is in

the middle (*DAB*) or end (*CDA*). In general, the results of Experiment 2 lend weight to the belief that plants tested in the morning suffer greater damage than when tested during other periods of the day or night.

TABLE II

SUMMARY OF ANALYSES OF VARIANCE FOR EXPERIMENTS 1 TO 6

	Experiment 1		Experiment 2			
	D.f.	Variance (12 hr.)	D.f.	Variance (6 hr.)	Variance (12 hr.)	Variance (18 hr.)
Varieties	21	206.1	24	1939.8	765.4	294.3
Exposure periods	1	3290.0	1	33172.4	4309.4	1775.0
Varieties × exposure periods	21	92.0	24	311.2	203.0	105.7
Replicates Replicates × periods	4	10.2	4	3426.6	3834.0	483.6
Residual	84	85.1	96	292.2	202.1	181.0
Total	131		149			

	Experiment 3		Experiment 4		Experiment 5		Experiment 6	
	D.f.	Variance	D.f.	Variance	D.f.	Variance	D.f.	Variance
Varieties	.74	443.9	74	516.7	24	1109.4	74	1106.3
Exposure periods	2	6237.0	2	13364.6	3	2409.3	2	33124.5
Varieties × exposure periods	148	392.3	148	298.9	72	245.1	148	285.5
Replicates Replicates × periods	3	3336.2	3	2590.6	4	2761.0	3	946.0
Residual	222	285.2	222	343.4	96	221.4	222	205.9
Total	449		449		199			

In experiments 3 to 6, the number of replicates was the same for each period involved. Experiments 3 and 4 are identical, except for the time of year, and the results agree in all essentials. The greater damage, in general, suffered by the plants in Experiment 3 is undoubtedly a result of the fact that they were less sturdy than those in Experiment 4. Differences in light conditions in the greenhouse between February and May were largely responsible.

The results of Experiment 5 are in conformity with the view that the *A* period is most damaging, but at variance with those of Experiment 2 (12- and 18-hr.) in that *DA* is more harmful than *AB*. The relatively small effect of the *A* period in Experiments 4 and 5 may well be due to the early hour of sunrise during the month of May, which tends to shift the *A* period towards *D*.

Experiment 6 provides results of considerable interest, and warrants the speculation that were the day to be further subdivided, the early part of the *A* period would be found to cause greater damage than the later part.

It is apparent from the results of statistical analyses recorded in Table II that observed differences between exposure periods are highly significant.

Differences in the reactions of varieties are clearly established in Experiments 2, 5, and 6, and suggested in the others. There was, however, little evidence of consistency in varietal reactions from one experiment to another. Under these circumstances, it is not surprising that no relation between the results of artificial drought tests and yield under field conditions could be established.

Discussion

That the time of day during which wheat plants are exposed to artificial drought has an important bearing on the results is established beyond a doubt. The marked susceptibility to treatment during the morning, and the development of resistance later in the day remains unexplained. There can be little doubt that light conditions are responsible for the differences observed; but discussion of the physiological processes involved cannot, at present, be justified.

Notwithstanding the varied response of different varieties to treatment, it is unlikely that the machine will be of use in selecting for resistance to drought. The results have failed to show any clear relation with yield under field conditions.

Acknowledgments

The authors are indebted to Dr. C. H. Goulden, Dominion Rust Research Laboratory, Winnipeg, and to Dr. J. W. Hopkins, Division of Biology and Agriculture, National Research Council, Ottawa, for advice in connection with experimental design and the statistical analysis of the results.

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POLARIMETRIC DETERMINATION OF STARCH IN CEREAL PRODUCTS

I. RAPID DETERMINATION OF STARCH IN CRUDE GLUTEN[1]

By K. A. Clendenning[2]

Abstract

Neither the original Mannich–Lenz procedure nor its recently proposed revisions proved suitable for the polarimetric determination of starch in gluten. The principal defect lay in the means of removing or correcting for proteins dissolved along with the starch and there also was a need for improvements in technique of starch dispersion.

In the present method, the starch of the gluten sample is dispersed by 15 min. boiling in a calcium chloride solution having a specific gravity of 1.30 and a pH of approximately 2.5. Frothing is controlled by the addition of one or two drops of *n*-octyl alcohol, constant volume being maintained during boiling by the addition of water. The proteins dissolved by this treatment are precipitated with 20% stannic chloride prior to filtration and polarization. This rapid procedure underestimates the starch content but the application of a factor to correct for the constant ratio of underestimation allows the real starch content to be determined with an accuracy of ± 0.5%.

Introduction

In connection with investigations on the separation of wheat flour into starch and gluten, a rapid method of determining the starch content of crude glutens was required as a means of assessing the value of different washing procedures. From the standpoint of simplicity and rapidity, polarimetric methods appeared preferable to the diastatic and gravimetric methods, and of the numerous polarimetric techniques that have been described, that of Mannich and Lenz (7) offered numerous advantages, particularly as revised by Hopkins (2). The Mannich–Lenz procedure and its recently proposed revisions accordingly have been applied to the rapid determination of starch in crude gluten. On introducing certain modifications to overcome difficulties with frothing and proteins dissolved along with the starch, a satisfactory method has been developed from the original procedure.

Experimental

The revision proposed by Jirak (5) proved out of the question since the magnesium chloride solution prescribed as starch solvent caused the gluten to become extremely viscous. The original procedure (7) also could not be applied to the determination of starch in gluten because of the large amounts of protein dissolved along with the starch and the lack of an accurate means of correcting for them. Hopkins (2) overcame the soluble protein difficulty

[1] *Manuscript received May 21, 1942.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as paper No. 195 of the Associate Committee on Grain Research and N.R.C. No. 1065.

[2] *Biochemist.*

in the analysis of wheat flour by thorough extraction of the flour with 65% ethyl alcohol prior to extraction with boiling calcium chloride solution. This revision proved inadequate for the determination of starch in gluten, low inconsistent results always being obtained because of the large amounts of protein that were dissolved despite careful preliminary alcoholic extraction. Protein precipitants were therefore tested experimentally by the writer, phosphotungstic acid being chosen because of its wide use in polarimetric methods of starch determination (1, 6, 9, 10, 11) and stannic chloride because Mannich and Lenz (7) claimed that in contrast to other protein precipitants, this reagent would precipitate proteins satisfactorily without precipitating starch.

Before dealing with the removal of proteins from calcium chloride solutions containing starch, attention should be directed to difficulties that have been experienced by a number of analysts in applying the revised Mannich–Lenz procedure to the determination of starch in cereal products (3, 4, 8). Various suggestions have been made as to the heat treatment and acidity that should be employed to obtain satisfactory starch dispersal. From experimental studies to be reported in detail in a second paper, the writer has reached a number of conclusions concerning the proper conditions for the extraction and dispersal of wheat starch by boiling calcium chloride solution. For the present it will suffice to note the following relevant points.

1. The stock calcium chloride solution should have a specific gravity of 1.30 and a definitely established acidity. Prior to use, its pH must be adjusted to between 2.0 and 3.0, preferably 2.5.

2. Boiling is best conducted in 300 to 400 cc. Berzelius beakers. It should be made to proceed steadily for at least 15 min., constant volume being maintained by the addition of water.

3. To control frothing, one or two drops of *n*-octyl alcohol should be added to the calcium chloride solution.

The Precipitation of Soluble Proteins

A gluten sample known to contain 10.3% starch was ground to pass a 140 mesh sieve and a series of 2-gm. subsamples then was extracted by 15 min. boiling with 60 cc. stock calcium chloride solution + 2 cc. 0.8% acetic acid. To the cooled extracts, volumes of freshly prepared 10% phosphotungstic acid and 20% stannic chloride ranging from 0 to 15 cc. were added, after which the extracts were made to volume, mixed, filtered, and polarized in 1 dm. tubes. The protein control of the filtered extracts was determined by Kjeldahl nitrogen estimations on 25 cc. aliquots, employing a factor of 5.7 to convert nitrogen to protein content. The measurements of optical rotatory power and protein content are given in Figs. 1 and 2.

From the data presented here, it is clear that stannic chloride will remove dissolved proteins quite as efficiently as the more widely used phosphotungstic acid. The stannic chloride reagent has a decided advantage in that it does not precipitate starch when present in excess. Phosphotungstic acid will, in

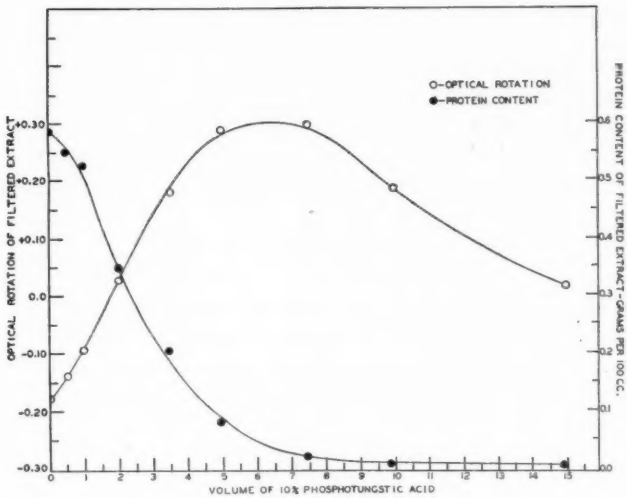
O—OPTICAL ROTATION
●—PROTEIN CONTENT
OPTICAL ROTATION OF FILTERED EXTRACT
PROTEIN CONTENT OF FILTERED EXTRACT-GRAMS PER 100 CC.
VOLUME OF 10% PHOSPHOTUNGSTIC ACID

FIG. 1. *The effect of increasing volumes of 10% phosphotungstic acid upon the optical rotatory power and protein content of calcium chloride extracts of gluten.*

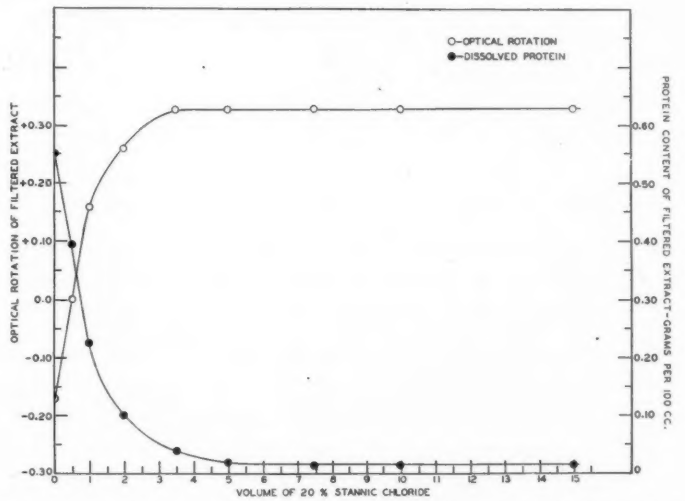
O—OPTICAL ROTATION
●—DISSOLVED PROTEIN
OPTICAL ROTATION OF FILTERED EXTRACT
PROTEIN CONTENT OF FILTERED EXTRACT-GRAMS PER 100 CC.
VOLUME OF 20 % STANNIC CHLORIDE

FIG. 2. *The effect of increasing volumes of 20% stannic chloride upon the optical rotatory power and protein content of calcium chloride extracts of gluten.*

fact, precipitate all of the starch dispersed in calcium chloride solution if added in sufficient quantity. The only undesirable effect of 20% stannic chloride consists of a tendency for gluten extracts to become cloudy on standing, when this reagent is present in large excess. As stannic chloride is highly acid in aqueous solution, starch also is apt to be degraded on standing

over long periods in its presence. Between 3.5 to 5.0 cc. 20% stannic chloride should be added to calcium chloride extracts of 2-gm. gluten samples for the satisfactory removal of dissolved proteins, judging from the data of Fig. 2. Using 3.5 cc. 20% stannic chloride upon calcium chloride extracts of 2-gm. samples of an absolutely starch free gluten, crystal clear filtrates having an optical rotation of exactly zero were obtained. This volume of stannic chloride accordingly has been adopted as a standard in the routine analysis of gluten.

The Effect of Gluten Particle Size upon Starch Extraction

The extraction of starch from gluten particles is rendered difficult by the enmeshing of the starch grains in the proteinaceous matter. Careful grinding of the material prior to extraction should facilitate the complete removal of the starch because of the resulting increase in surface. To test the effect of particle size, samples ground in the Wiley mill and ball mill were subjected to analysis by the rapid polarimetric method described below (Table I).

TABLE I

APPARENT STARCH CONTENT OF SAMPLES GROUND IN THE WILEY MILL AND BY BALL-MILLING

Sample	Wiley-milled		Ball-milled	
	α	Apparent starch, %	α	Apparent starch, %
1	0.29°	7.25	0.37°	9.25
2	0.50°	12.50	0.58°	14.50
3	0.32°	8.00	0.35°	8.75
4	0.15°	3.75	0.21°	5.25

Samples that had been ground in the Wiley mill also were separated into different particle sizes by passing through a series of sieves, the separated fractions then being similarly analysed (Table II).

TABLE II

EFFECT OF PARTICLE SIZE UPON THE EXTRACTION OF STARCH FROM GLUTEN WITH CALCIUM CHLORIDE SOLUTION

Particle size	Gluten No. I		Gluten No. II		Gluten No. III	
	α	Apparent starch, %	α	Apparent starch, %	α	Apparent starch, %
Passing:						
140 mesh	0.59°	14.75	0.45°	11.25	0.33°	8.25
85-100 mesh	0.55°	13.75	0.40°	10.00	0.29°	7.25
36-60 mesh	0.40°	10.00	0.30°	7.50	0.19°	4.75
20-36 mesh	0.35°	8.75	0.26°	6.50	0.15°	3.75

The effect of particle size upon the amount of starch extracted by 15 min. boiling apparently is a very marked one. Subsequent tests showed that this effect as it appears in Table II is not caused by differences in the starch content of various sized particles. From this it is clear that grinding the samples to pass a 140 mesh sieve should be adopted as a standard practice in the preparation of all samples for analysis.

Effect of Extraction Time upon Starch Recovery

The foregoing data, as well as showing the effect of particle size upon the amount of starch extracted in a 15 min. extraction period, indicated that the starch content of gluten ground to pass 140 mesh might be underestimated through incomplete extraction. To test the effect of extraction time upon starch recovery, duplicate 2-gm. samples of a series of 140 mesh gluten samples of known starch content were extracted for 15, 40, and 60 min., removing the dissolved proteins as usual with stannic chloride. The polarimetric readings are recorded below in Table III.

TABLE III

EFFECT OF EXTRACTION TIME UPON THE COMPLETENESS OF STARCH RECOVERY FROM GLUTENS OF KNOWN STARCH CONTENT

Sample No.	Optical rotation for 100% recovery	Optical rotation with 15 min. extraction	Optical rotation with 40 min. extraction	Optical rotation with 60 min. extraction
1	+ 0.18°	0.15°	0.15°	0.17°
2	+ 0.36°	0.30°	0.33°	0.33°
3	+ 0.55°	0.45°	0.51°	0.52°
4	+ 0.73°	0.60°	0.71°	0.71°

In assessing the significance of the data of Table III the effect of increased duration of boiling upon several different factors contributing to the extract's optical activity must be borne in mind. The precipitation of the proteins dissolved by 15 min. extraction is not absolute, sufficient remaining (0.04 gm. per 100 cc.) to cause a negative reading of 0.003°, and with a longer period of boiling, this error should tend to increase. Increased duration of boiling also should reduce the specific rotation of wheat starch judging from the data of Mannich and Lenz (7). These two effects, however, should not cause a negative error totalling more than −0.01°. There remains the possible occlusion of starch on the stannic chloride–protein precipitate. In view of the large amount of protein precipitated, a small attending loss of dissolved starch is regarded as inevitable.

Because of the possible effects of residual dissolved proteins, altered specific rotation with prolonged boiling, and starch losses attending the removal of

proteins, it is not surprising that the optical rotatory power of extracts obtained by 60 min. boiling is below the theoretical values given to represent 100% recovery. The values in fact are low by 0.01 to 0.03° in the 60 min. extraction series. This small bias is attributed largely to starch occlusion on precipitated proteins. With 15 min. extraction, the optical rotation values are low by 0.03–0.13°, depending on the starch content of the sample. This increased discrepancy is attributed to an incomplete extraction of the starch.

Since the operator must devote almost continuous attention to samples during their extraction in stirring, maintaining constant boiling rate and volume, and rubbing down the sides of the beakers, an extraction period of more than 15 min. increases the labour required per determination very considerably. Lengthening the procedure in order to reduce the correction factor is in the author's opinion unwarranted for the purposes of routine analysis. In the rapid procedure outlined below, in which 15 min. extraction is used in conjunction with a correction factor, an experienced technician should be able to determine the starch content of 12 or more gluten samples in six hours with little difficulty.

Calculation of the Ratio of Underestimation of Starch Content

For this purpose, several hundred grams of gluten was completely freed of starch grains, as judged by microscopic observation, through careful and prolonged machine and hand washing. After air-drying and grinding to pass 140 mesh, calcium chloride extracts of this material failed to give a starch test with iodine. The dry finely ground gluten was then divided into five subsamples and to these wheat starch of known purity was added to give mixtures containing 0 to 18% starch. After careful mixing in the dry state, distilled water was added slowly with continuous stirring until a lump of moist dough was obtained containing all of the gluten and starch of the subsample. After careful kneading for half an hour in the dough state, the mixtures were drum-dried and ground again to pass a 140 mesh sieve. The weight of starch added to each subsample, less known impurities, was used in calculating the *actual* starch content of each sample. The *apparent* starch content was determined polarimetrically, employing the equation of Mannich and Lenz to convert the observed reading of optical rotatory power to starch content (Fig. 3).

The relationship of the apparent to the actual starch content is seen to be linear over the range 0 to 18% starch content and is represented by the equation : % present = 1.21 (% found). Because of the constancy of the ratio between the actual and apparent starch content, the rapid determination of the latter should give an accurate measure of the true starch content when multiplied by the factor 1.21. Employing this correction factor, analyses were conducted on two glutens prepared in another laboratory by the method given above so as to contain 8 and 16% starch, respectively. Agreement between the actual and determined starch content then was within $\pm$ 0.3%. The starch content of a crude machine-washed gluten was determined by a diastatic technique which took full cognizance of the effect of the gluten upon

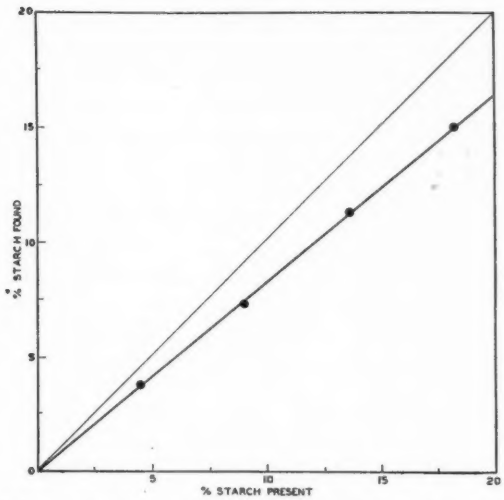

FIG. 3. *Relation of actual starch content of crude glutens to starch content determined polarimetrically.*

the final sugar determinations. Agreement within 0.3% was again observed when this gluten was analysed by the rapid technique described below.

Method

Reagents

(1) Concentrated calcium chloride solution

Dissolve two parts of $CaCl_2.6H_2O$ in one part of water. Adjust the specific gravity to 1.30. Adjust the pH to 5.50 with dilute hydrochloric acid and sodium hydroxide. In preparing this stock solution only reagent quality chemicals should be used. Filter with suction until crystal clear before using.

(2) 20% Stannic chloride solution

Dissolve 20 gm. $SnCl_4.5H_2O$ by warming with about 80 cc. of the above calcium chloride solution. Cool and make to volume with stock calcium chloride solution in a 100 cc. volumetric flask. After mixing and filtering, this reagent should be crystal clear. Unlike many protein precipitants, this reagent does not have to be freshly prepared before use.

(3) 0.8% Acetic acid

(4) *n*-Octyl alcohol

(5) 95% Ethyl alcohol

Procedure

Weigh 2.0 gm. of the dry 140 mesh gluten into a 300 to 400 cc. Berzelius beaker. Add 60 cc. calcium chloride solution and 2 cc. 0.8% acetic acid, omitting the initial treatment with water required in the analysis of cereal

products of high starch content. Immediately whip the contents of the beaker gently until a uniform suspension is obtained. All lumps must be completely disintegrated at this stage. Add two drops *n*-octyl alcohol for frothing control. After marking the level of the beaker's contents with a wax pencil, bring to boiling in approximately five minutes, stirring constantly. It is our practice to use a 3 in. non-luminous flame of the Cenco Tirril type burner, protected by an earthenware burner guard.

When boiling is proceeding briskly, reduce the flame by one-half inch and maintain gentle but steady boiling for 15 min. During this period of boiling, the mixture should be stirred occasionally, taking care at all times to keep the gluten particles rubbed down into the solution from the sides of the beaker by the use of a rubber tipped stirring rod. Water should be added with stirring from time to time so as to maintain a constant boiling volume. After 15 min. boiling, cool to room temperature in running tap water. Pour the contents of the beaker into a 100 cc. volumetric flask containing 3.5 cc. 20% stannic chloride solution. After shaking the contents of the volumetric flask, complete the transfer by successively rinsing the beaker with stock calcium chloride solution. Destroy any foam by adding one or two drops of 95% ethyl alcohol. Now make to volume accurately with the calcium chloride solution. Shake vigorously for three minutes. Allow 10 cc. to run through a fluted 15 cm. Whatman No. 12 filter paper, taking care to wet the whole filtering surface thoroughly. Discard this first filtrate, and then filter the remainder of the solution without suction. Polarize the crystal clear filtrate in a 1 dm. tube. In the present work, polarization has been by means of an Adam Hilger triple-field polarimeter, using either a Zeiss sodium vapour lamp or an Adam Hilger F289 sodium burner as a source of sodium light. The starch content of the crude gluten can then be calculated from the formula $\frac{(50\ \alpha)\ (1.21)}{(\text{wt. of sample in gm.})} = \%$ starch, where α = observed optical rotation in degrees.

Acknowledgments

The author is indebted to Dr. W. H. Cook, Dr. G. A. Adams, and Dr. J. W. Hopkins for numerous helpful suggestions. It is also a pleasure to acknowledge the assistance of Mr. D'Arcy Wright throughout the present work.

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INTERFERTILITY STUDIES AND INHERITANCE OF LUMINOSITY IN *PANUS STYPTICUS*[1]

By Ruth Macrae[2]

Abstract

Examination of five collections of *Panus stypticus* (Bull.) Fries from Europe and of 10 collections from North America has corroborated the findings of other workers that the European form of this fungus is non-luminous and the American form luminous. Series of pairings in all possible combinations of monosporous mycelia from single fruit bodies have shown that both forms are heterothallic and tetrapolar. Pairings between monosporous mycelia of different collections of the American and European forms are fertile. A study of diploid mycelia and fruit bodies of the F_1 generation from crosses between the luminous and non-luminous forms, and of haploid mycelia from an F_1 fruit body produced by such a cross, has shown that luminosity in this species is an inherited character, that it is governed by a single pair of Mendelian factors in which luminosity is dominant over non-luminosity, and that the luminosity factors form all possible combinations with the interfertility factors. A point of special interest in the study of the inheritance of this factor is in its expression as a dominant character in the dikaryotic mycelium and fruit bodies of the F_1 generation.

Since the discovery of heterothallism in the Basidiomycetes, genetical studies in these fungi have dealt almost exclusively with the sexuality or interfertility factors and related problems. Although in more recent years much work has been done on the inheritance of morphological and pathological characters and the origin of physiologic races through mutation and hybridization in the Uredinales[3] and Ustilaginales[4], in the Hymenomycetes the inheritance of factors other than the interfertility factors has been studied in only a few species. The work that has been done in the latter group along this line is reviewed briefly here.

Vandendries and Brodie (52) and Brodie (5) have reported on what they have called the "barrage" phenomenon in *Lenzites betulina* (L.) Fr., a tetrapolar species. They found that in this and other species when certain haploid[5]

[1] *Manuscript received May 11, 1942.*

Contribution No. 705 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. Part of a thesis presented to the School of Graduate Studies, University of Toronto, Toronto, Ont., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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[3] *Buller (7, 8); Craigie (10); Gordon and Welsh (18); Gassner and Straib (16); Johnson and Newton (22, 23, 24, 25); Johnson, Newton, and Brown (26, 27); Levine and Cotter (32); Newton and Johnson (37, 38, 39); Newton, Johnson, and Brown (40, 41); Stakman, Levine, and Cotter (49, 50); Waterhouse (53, 54).*

[4] *A review of the genetics of the Ustilaginales with a list of references is given by Christensen and Rodenhiser (9).*

[5] *In the heterothallic Basidiomycetes the mycelium arising from a single spore is referred to as a monosporous, haploid, or monokaryotic mycelium or a haplont since it possesses nuclei with the single or haploid number of chromosomes arranged singly, that is as monokaryons, within the cells. The mycelium produced by the union of two compatible haploid mycelia is called a diploid or dikaryotic mycelium or a diplont because the haploid nuclei which it contains are associated together in pairs or dikaryons within the cells and each cell, therefore, contains the diploid or double number of chromosomes. An explanation of the use of these and other associated terms has recently been given by Buller (7, 8).*

mycelia were grown side by side on nutrient agar, the two mycelia exhibited a mutual aversion resulting in the formation of a zone or barrage between them which was bridged by only a few hyphae or none at all. The authors found that the presence of the barrage in *L. betulina* was correlated with the genetic constitution of the mycelia concerned and appeared when one pair of the interfertility factors, designated by the authors as the *Bb* factors, was unlike. Its manifestation was, on the other hand, completely independent of the other pair of interfertility factors.

Biggs (4), examining the pairing reactions of 25 monosporous mycelia of a single fruit body of *Cytidia salicina* (Fr.) Burt, observed that both the barrage phenomenon and the formation of false clamp connections in this species occurred when the paired mycelia possessed in common one of the interfertility factors, arbitrarily designated by her as the *B* or *b* factor. In this species the factors governing the formation of a barrage and those controlling the production of false clamp connections are evidently both associated with the same pair of interfertility factors.

Quintanilha (44) in *Coprinus fimentarius* Fr. and Routien (48) in *Coprinus plicatilis* Fr. found that false clamp connections were formed in these species when the *A* or *a* factors of the paired monosporous mycelia were different and the *B* and *b* factors alike.

Kaufert (28) noted four types of reactions between haploid mycelia from single sporophores of the tetrapolar species *Pleurotus corticatus* Fr. These types of reactions were correlated with the similarity or dissimilarity of the interfertility factors in the paired haplonts. In the compatible pairings where the mycelia contained both sets of interfertility factors ($AB \times ab$, $Ab \times aB$) and in the incompatible pairings where the mycelia had the same pair of interfertility factors ($AB \times AB$, $ab \times ab$, $Ab \times Ab$, $aB \times aB$) there was a complete intermingling of the mycelia of the two haplonts at the line of junction between them. In the incompatible pairings where the *A* factors were the same and the *B* factors different ($AB \times Ab$, $ab \times aB$) a sharp line of demarcation, consisting of a narrow white line of aerial mycelium, was produced where the hyphae of the two mycelia mingled but did not grow further into the area occupied by the other haplont. Where the *B* factors were the same and the *A* factors different ($AB \times aB$, $ab \times Ab$), the hyphae of the two mycelia intermingled freely and anastamoses occurred but there was an almost complete inhibition of coremial and conidial formation in both mycelia.

In all these investigations the characters described were found to be closely associated with the interfertility factors. In the investigations reviewed below, other characters have been shown to form all possible combinations with the interfertility factors and the dominance of the character or its allelomorph to be manifested when the two nuclei became associated as a dikaryon in the diploid mycelium.

Zattler (55) paired with each other haploid mycelia of two varieties of *Collybia velutipes* (Curt.) Fr., one with dark brown and the other with white mycelium. He found that the diploid mycelium formed by the paired

haplonts was brown in colour and that the haplonts isolated from a fruit body produced by the diploid mycelium were of four kinds: dark brown, light brown of two different intensities, and white. From his observations he concluded that the colour of the mycelium was governed by two pairs of Mendelian factors *Rr* and *Vv* which formed all possible combinations with the interfertility factors. The presence of either of the two dominant factors, *R* or *V*, gave to the mycelium a brown coloration but *R* gave a more intense colour than *V*, hence *Rv* haplonts were intense brown, *Rv* and *rV* haplonts light brown of different intensities, and *rv* haplonts white.

Working with a Canadian strain of *Schizophyllum commune* Fr., the same author found that when certain haploid mycelia were paired the diploid mycelium produced abnormal ball-shaped fruit bodies, "Knäuel-Fruchtkorper", but when other haploid mycelia of the same strain were paired the diploid mycelium gave rise only to normal fruit bodies. He judged from his experiments that in this Canadian strain normal fruit body formation was governed by a single pair of Mendelian factors *Gg*, the factor *G* for normal fruit body formation being dominant over the factor *g* for abnormal fruit body formation.

Gilmore (17) found that in *Psilocybe coprophila* Fr. the production of abnormal fruit bodies of various types was more preponderant in two of the crosses than it was in other crosses of monosporous mycelia from a single fruit body. She came to the conclusion that this character was inherited but could not be expressed in a simple Mendelian ratio.

Dickson (11, 12, 13) made a study of the morphological characters of haploid and diploid mycelia of *Coprinus sphaerosporus*, their growth rates and the production of fruit bodies in culture. He found that the F_1 generation from any cross consisted of several types with respect to these characters and from his experiments came to the conclusion that one of the types "is of the original 'wild type' and contains the full chromosome complement combined with a large percentage of dominant genes". The other types found, he suggests, may have contained a number of recessive factors and/or certain chromosome abnormalities such as translocations, deletion, or inversion which resulted in morphological and physiological types different from both parents.

Quintanilha and Balle (45, 46, 47) found that some of the spores of certain fruit bodies of *Coprinus fimentarius* gave rise to vigorous mycelia with abundant branching and fairly rapid rate of growth, and others to poorly developed mycelia varying from a single short hypha or a small swelling to a vesicular, branched mycelium of very slow growth. They called these two types "normal" and "dwarf" mycelia. Among the fruit bodies producing normal and dwarf mycelia, they found one in which every tetrad examined (101 in all) produced two normal and two dwarf mycelia. When two normal haploid mycelia from this fruit body were paired, only normal mycelia were produced in the next generation; a dwarf mycelium paired with a normal mycelium gave rise to two normal and two dwarf mycelia in each tetrad of the next generation; pairings of two dwarf mycelia never succeeded. They explain these results by the presence of a pair of Mendelian factors *Nn*,

where N is responsible for normal growth and n is its sublethal allelomorph. They have remarked that in the dikaryophase N is completely dominant over n and the heterozygous diploid mycelia Nn and the fruit bodies that they produce are indistinguishable from the homozygous dominants NN. They were not able to show an association of the factors for dwarfness with the interfertility factors but found that these factors were distributed equally among the four interfertility groups and concluded that the three pairs of factors must be situated on three different pairs of chromosomes.

From their observations on dwarfness in *C. fimentarius* Quintanilha and Balle are convinced of the existence of a series of multiple allelomorphs (N, n, n_1, etc.) of sublethal genes, each one responsible for a special type of dwarfness. In two fruit bodies they found evidence of irregular disjunction: in addition to the numerous tetrads of two normals plus two dwarfs, many others gave three dwarfs and one normal, one dwarf and three normals, four more or less dwarf, or four nearly normal. Genetical analysis of this type of dwarfness was not finished when they published their results but they concluded that it was a case of hereditary dwarfness probably conditioned by a single pair of Mendelian factors but varying greatly in its manifestation due to environmental conditions or the arrangement of the chromosomes within the cell itself.

While working with *Peniophora Allescheri* Bres., Nobles (42) obtained a mutant haploid mycelium which was entirely unlike the normal haploid mycelium in rate and type of growth and production of conidia. The normal mycelium was slow growing with scanty aerial hyphae and numerous conidiophores; the mutant mycelium had a rapid rate of growth, abundant aerial hyphae, and lacked conidiophores. When the mutant mycelium was paired with a normal haploid mycelium the diploid mycelium produced was like the mutant parent in its rapid rate of growth and abundant aerial hyphae but like the normal parent in that conidiophores and conidia were produced. It was found that the conidia of this species had only one nucleus and so in order to ascertain if the nuclei of both parent types were present in the hybrid mycelium, 295 mycelial colonies from the conidia of the hybrid mycelium were examined; 153 of these were found to be identical with the mutant parent and 142 with the normal parent. In this species, "the two nuclei of a pair, though existing independently in the cells, coöperate in determining the characters of the diploid colony".

In the investigations described in the present paper an additional example is given of the inheritance of a dominant character in the Hymenomycetes. The fungus studied is *Panus stypticus* (Bull.) Fries, a small coriaceous agaric which grows in groups or clusters usually on the wood of deciduous trees and very rarely on coniferous hosts. Collections of this fungus from North America differ from those of European origin in that the former are luminous and the latter non-luminous. Polysporous cultures and series of monosporous cultures were obtained from the fruit bodies of collections from North Amrica and Europe, the fruit bodies and cultures examined for luminosity, and the

different series of monosporous isolates paired by themselves and with each other in order to determine the relationship between the luminous and non-luminous forms of the fungus and the inheritance of the factor for luminosity in a cross between them.

Materials and Methods

A list of the collections from which cultures were made, together with the hosts and localities from which they came, is given in Table I.

TABLE I

Collection number	Host	Locality
American		
1532	*Quercus rubra*	Timagami, Ont.
2189	*Acer* sp.	Ann Arbor, Mich.
2190		Ste. Anne de Bellevue, Que.
2191		New Haven, Conn.
5105	*Alnus incana*	Ottawa, Ont.
6004	*Betula papyrifera*	Lake MacDonald, Que.
7543	*Acer* sp.	Chelsea, Que.
7544	*Alnus incana*	Eardley, Que.
8303	Deciduous stump	Otter Lake, near Huberdeau, Que.
9518	*Alnus incana*	Ottawa, Ont.
European		
1667	*Fagus* sp.	Beiersdorf, near Dresden, Germany
2118	*Quercus pedunculata*	Darmstadt, Germany
2188	*Salix* sp.	Sigless, Burgenlande, Austria
5120	*Quercus* sp.	Potsdam, Germany
5122		Wageningen, Holland

Polysporous and monosporous cultures were obtained from single fruit bodies of each of these collections in the following manner. A fruit body was moistened and suspended over a sterile glass slide. From the spore deposit thus obtained, spore dilutions in sterile distilled water were made and spread over the surface of a gelatine medium, containing 10% gelatine, 1% lactose, and 1% dextrose, in Petri dishes. When the spores had germinated, monosporous cultures were isolated by cutting out, with a fine needle under the compound microscope, a square of gelatine containing a single sporeling and placing it in a tube of malt agar. Polysporous cultures were obtained by transferring a square of gelatine containing a number of germinating spores to a tube of malt agar.

Most pairings were made by placing small pieces of two monosporous or haploid mycelia about one centimetre apart on slants of malt agar in test-tubes; some, however, were made in Petri dishes containing malt agar. Two weeks or more after inoculation the mycelium at the junction of the two haploid mycelia was removed, mounted in a 7% aqueous solution of potassium hydroxide, stained with an aqueous solution of phloxine, and examined for clamp connections.

In the accompanying tables of pairings, with the exception of the table in Fig. 9, the sign in each square represents the result of a single pairing between two haploid mycelia. The plus sign indicates that the two haploid mycelia had united to form a diploid mycelium with clamp connections, i.e., the mycelia were interfertile or compatible. The minus sign indicates that no clamp connections were found in the pairing, i.e., the two mycelia were intersterile or incompatible. The table in Fig. 9 differs from the other tables in that the sign in each square represents the results of a series of pairings between a number of haploid mycelia from two different collections, rather than of a single pairing between two haploid mycelia.

Whether the cultures and fruit bodies were luminous or non-luminous was determined by examining them after 15 to 30 min. in a dark-room when the eyes had become accustomed to the darkness and able to perceive a dim light.

Luminosity

Probably the first record of luminosity in *Panus stypticus* was made by Ellis (15) who writes: "Some time last fall (1885) Prof. Thos. G. Gentry, of Philadelphia, Pa., called my attention to the fact that *Panus stypticus*, Fr., is phosphorescent. Prof. G. had collected some specimens of this species and laid them with other fungi on a shelf to dry. On examining the specimens the same evening, it was found that the gills of the Panus were distinctly phosphorescent, a fact which I have been able to verify by my own observation of specimens, soon after collected at Newfield. By careful examination, the luminosity was found to proceed from the gills and not from the stipe, nor from the upper surface of the pileus, nor, finally, as was at first suspected, from any fragment of rotten wood attached to the specimen. This phosphorescence was not observed in all specimens brought in for examination, and seemed to depend on some peculiar condition of the air, having been noticed only in specimens gathered in damp weather or just before a storm."

Atkinson (3), Hard (20), Murrill (36), and Kauffman (29) describing collections of *P. stypticus* found in North America, state that the fungus is "phosphorescent". Molisch (35), commenting on the findings of Gentry and Ellis, says that he had collected *P. stypticus* repeatedly around Vienna, Austria, and had never seen luminescence in the fruit bodies, nor were pure cultures of the mycelium on bread luminous. Miss Johnson (21) working in England on the biology of *P. stypticus* reported that no fruit bodies that she had examined were luminous.

Buller began his observations on the luminosity of *P. stypticus* in 1910 and published in 1924 a full account of his investigations. He reports that all the collections from North America that he had examined were luminous, but that those from England were non-luminous. From his investigations he concludes that there are two distinct physiological forms of the fungus: one in North America having luminous fruit bodies which he calls *Panus stypticus luminescens*, and another in England having non-luminous fruit

bodies, to which he gives the name *Panus stypticus non-luminescens*. He says (6) that "Since luminous fruit-bodies of *Panus stypticus* have never been recorded by any European mycologist, it seems extremely probable that *P. stypt. non-luminescens* is not confined to England but occurs throughout Europe. Probably its distribution is Eurasian in extent. It seems most likely that one of the two physiological forms arose from the other and that to-day they are separated geographically because the great barrier of the Atlantic Ocean prevents their intermingling."

The writer has examined five collections of *P. stypticus* from Europe, three from Germany, one from Austria, and one from Holland (Table I). None of the fruit bodies of the five collections were luminous, nor were the polysporous or monosporous mycelia originating from them. These observations, in addition to Molisch's findings for collections from Vienna, agree with Buller's supposition that *P. stypticus* found on the continent of Europe, like the form found in England, is non-luminous.

Studying the luminosity of the American form of *P. stypticus* Buller found that the fruit bodies when immature were luminous all over their surfaces but when mature the luminosity was confined to the gills. He observes that "A fruit-body is most strongly luminous when it is just full-grown. Thereafter, as it exhausts itself by shedding spores its light gradually declines, finally becoming invisible shortly before the end of the spore discharge period." The fruit bodies of *P. stypticus* absorb moisture readily and may be revived after drying. Those that have not passed maturity and are still luminous lose this luminosity when dried but regain it when moistened. This occurs frequently throughout the growing season of the fungus and may explain the observation made by Ellis that "phosphorescence was not observed in all specimens brought in for examination and seemed to depend on some peculiar condition of the air, having been noticed only in specimens gathered in damp weather or just before a storm."

Buller noted that when wood collected with the fruit bodies was moistened and left for several hours or days the mycelium then growing at the surface was luminous, although "sometimes pieces of wood bearing fruit-bodies never showed the least sign of luminescence." (Compare Gentry's observation quoted above.) The writer has observed that if the wood on which the fungus is actively growing and producing fruit bodies is sufficiently moist, the mycelium in it is brightly luminous. It would seem that when fruit bodies and mycelium are physiologically active they are luminous, but if, through dryness or for any other reason, activity ceases, luminosity is curtailed.

In all 10 collections of *P. stypticus* from North America examined by the writer luminous fruit bodies were found. In six of the collections all the fruit bodies were luminous. In a seventh collection, No. 1532, the one fruit body examined was found to be luminous. Most of the fruit bodies of another collection, No. 2191, were luminous, a few were not. No attempt was made to obtain spores from the non-luminous fruit bodies of this collection but it is

possible that they were no longer luminous because their activity had ceased. In the two remaining collections, Nos. 7544 and 8303, both luminous and non-luminous fruit bodies were present, all actively shedding spores. It is not known if the non-luminous fruit bodies of these last two collections were nearing the end of their spore discharge period and so had lost their luminosity, although the abundance of spores given off would seem to preclude this possibility, or if the physiological vigour of the fruit bodies had been affected by adverse conditions and luminosity curtailed. That these fruit bodies were from a non-luminous strain seems unlikely since some of the fruit bodies of the same collection were brightly luminous and the partial recovery of luminosity by two of the non-luminous fruit bodies of collection No. 8303 suggests that the non-luminous fruit bodies of this collection may have been luminous originally. Moreover, at least some of the monosporous cultures and all the polysporous cultures derived from each of the non-luminous fruit bodies of the two collections were luminous. In the non-luminous strain from Europe, fruit bodies, monosporous cultures, and polysporous cultures are all consistently non-luminous.

Studying the luminosity of monosporous and polysporous mycelia of the American form of *P. stypticus* in culture, the writer has found that they gradually lose their luminosity with age and consequent loss of activity but regain it when transferred to fresh media. The monosporous cultures isolated from a single fruit body were found, even when first isolated, to vary in the intensity of light produced and a few monosporous cultures isolated from luminous fruit bodies were found to be non-luminous from the beginning. Many of the monosporous mycelia lost their luminosity permanently a few weeks or a number of months after isolation and only a comparatively few have kept it throughout the time of these experiments. All the polysporous cultures, however, have retained their luminosity up to the present time. The polysporous cultures of any fungus are generally more vigorous than the monosporous cultures and it would seem here that the more vigorous cultures have retained their luminosity.

To determine whether or not the spores were luminous, heavy spore deposits from three luminous fruit bodies on glass slides and another spore deposit from a brightly luminous fruit body on malt agar were examined in the dark but no trace of luminosity was visible. The spore deposit on malt agar was examined on the first and second days after germination of the spores had taken place but again no luminosity could be seen. Ten days after germination, however, the young mycelial colony was brightly luminous. The non-luminosity of the spores and young mycelia was noted by Buller who suggests that the very young mycelia may be luminous but so faintly so as to be invisible to the human eye.

A monosporous and a polysporous culture of collection No. 6004 of the luminous American form of *P. stypticus* and a diploid culture, XLIV, produced by pairing a luminous monosporous culture, No. 6004–6, with a non-luminous monosporous culture, No. 5122–20, from a European collection were given

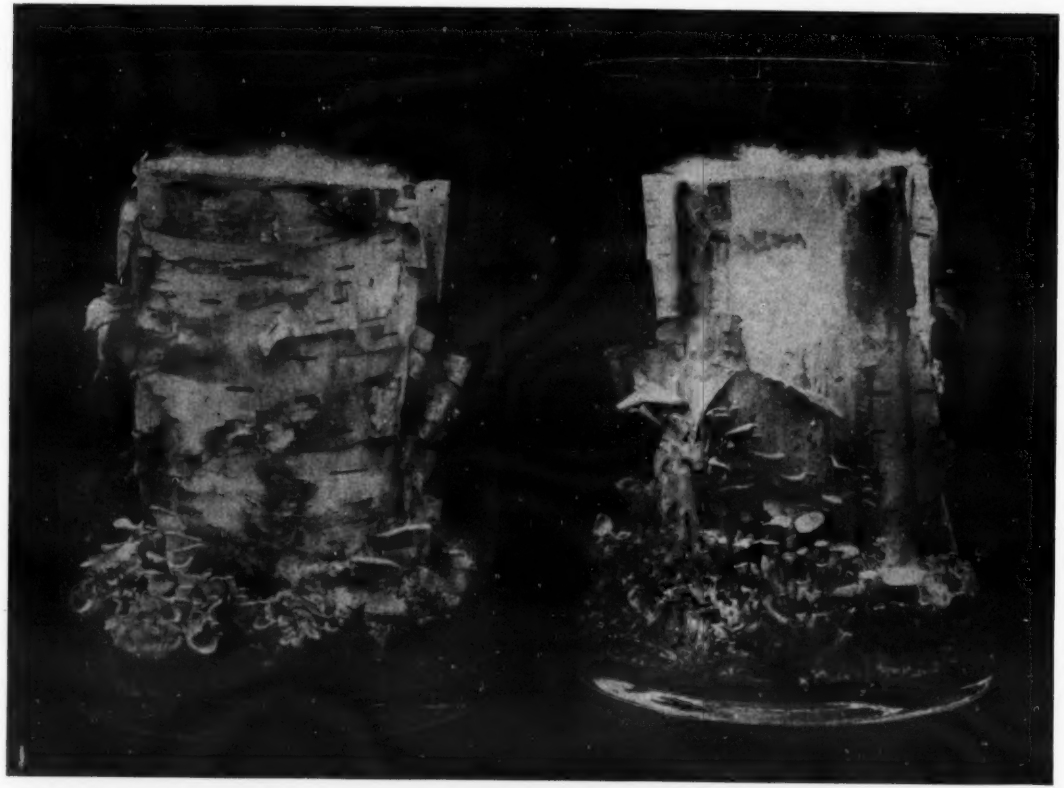
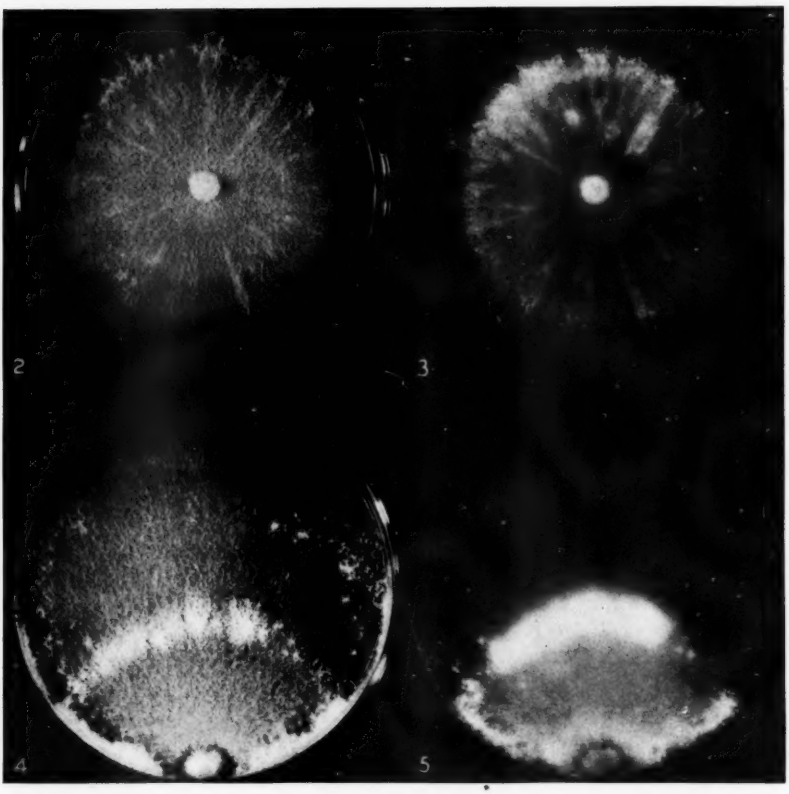


FIG. 1. *Panus stypticus. Hybrid fruit bodies produced by the diploid mycelium from a pairing between two monosporous mycelia of the luminous American and non-luminous European forms, 6004–6 × 5122–20, grown on a section of white birch resting on sphagnum moss in a wide-mouthed jar. Photographs show both sides of the same culture.* ×½.

PLATE I

PLATE II



Panus stypticus cultures grown on 1.25% malt agar. ×¾ approximately. FIG. 2. *A monosporous culture, No. 6004–6, five weeks old, photographed by reflected light.* FIG. 3. *The same culture as Fig. 2 photographed by its own light.* FIG. 4. *A polysporous culture, No. 6004, six weeks old, photographed by reflected light.* FIG. 5. *The same culture as Fig. 4 photographed by its own light.*

to Dr. L. E. Howlett of the Division of Physics and Engineering of the National Research Council of Canada. He very kindly made photographs of the spectra of the luminescence of all three cultures with a helium spectrogram for comparison, and reported concerning them: "Light was emitted from all three in the region 4900–5800 Å. Samples marked 6004–6 and 6004 exhibited much stronger fluorescence than the other one, XLIV, and showed a strong emission peak at about 5400–5500 Å. This peak was not nearly as apparent in sample XLIV. Samples 6004–6 and 6004 were given an exposure of 120 hours. Sample XLIV was given an exposure of 210 hours. A Zeiss high power spectrograph with an F2.3 aperture on the camera was used."

The spectrograms of these three cultures are reproduced in Plate IV. As was to be expected the three spectrograms are similar. They extend from near the beginning of the yellow region through the green and fade out in the blue green, with the peak in the yellow green region. These spectra are very like that photographed by Molisch (35) for the luminescence of the mycelium of an unidentified fungus, mycelium X. The spectrum for this fungus extends from 4800 to 5700 Å. (*P. stypticus*—4900 to 5800 Å.) The spectrum for the luminescence of *Armillaria mellea* on the other hand was found by Ludwig (33) to cover the region from 4600 to 5400 Å, i.e., from the middle of the yellow region through the green into the blue. It would appear that the wave length of the light given out by luminous fungi may vary for different species.

Photographs of the monosporous culture No. 6004–6 and the polysporous culture No. 6004, which were used for the helium comparison spectra, were taken by reflected light and in a totally dark room where the only light acting on the photographic plate was that of the luminous mycelium (Plate II). For the photographs of the cultures taken by their own light Wratten hypersensitive panchromatic plates and a lens aperture of F6.3 were used with an exposure of 26 and 50 hr. respectively.

Interfertility Studies

Series of pairings in all possible combinations of monosporous mycelia from single sporophores of collections of *P. stypticus* of North American and European origin have shown that both the luminous American and non-luminous European forms of this fungus are heterothallic and tetrapolar. These results were published by the writer in the Progress Report of the Dominion Botanist for the years 1931–1934 and in Nature (34) and have been confirmed by Vandendries (51) for the European form.

In Table II the results of pairings in all possible combinations of monosporous mycelia of three collections from North America and of five collections from Europe are summarized.

In Figs. 6 to 8 the results for the American collections Nos. 6004 and 5105 and the European collection No. 5122 are presented graphically. In these tables the four groups into which the monosporous cultures resolve themselves are designated by the factors *AB*, *ab*, *Ab*, and *aB*, which, following the usage

TABLE II

THE RESULTS OF SERIES OF PAIRINGS IN ALL POSSIBLE COMBINATIONS BETWEEN MONOSPOROUS CULTURES FROM SINGLE FRUIT BODIES OF *Panus stypticus*

Culture number	Age when paired	Number of cultures	Number of cultures				
			Group *AB*	Group *ab*	Group *Ab*	Group *aB*	Group *ab* + *aB*
American							
1532	38 days	9	3	3	1	2	
3105	62 days	22	10	8	3	0	1
6004	79 days	28	7	4	8	9	
European							
1667	52 days	9	5	1	2	1	
2118	41 days	9	4	3	1	1	
2188	65 days	10	2	4	3	1	
5120	29 days	27	4	4	12	7	
5122	1 year 213 days	13	3	3	5	2	

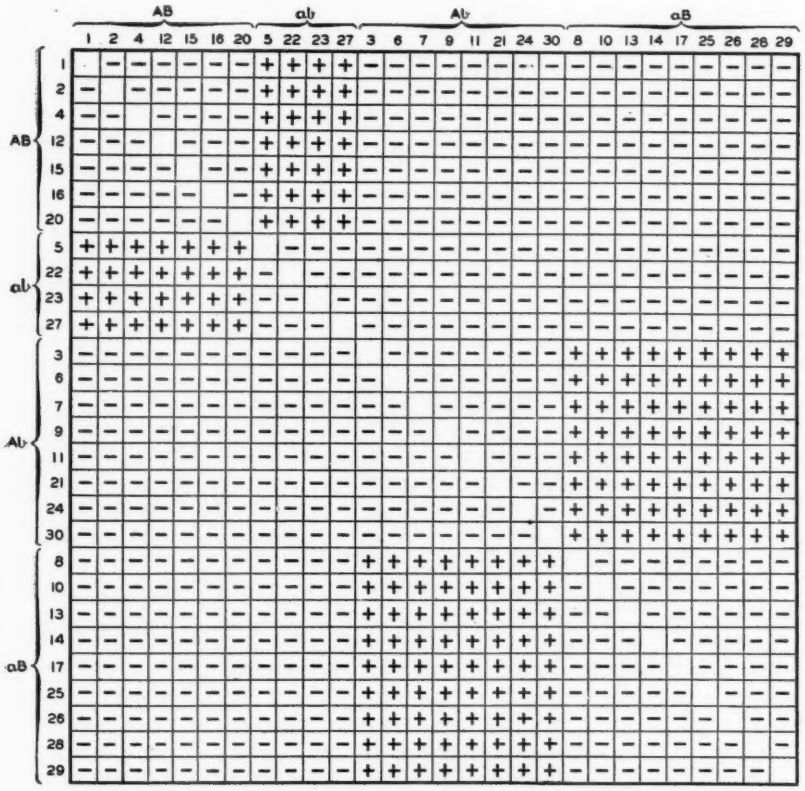


FIG. 6. *Table showing the results of pairing, in all possible combinations, a series of 28 monosporous cultures from a single fruit body of an American collection of Panus stypticus, No. 6004.*

		A'B'			a'b'			A'b'					a'B'	
		1	10	19	12	14	15	21	22	23	24	26	13	20
A'B'	1		−	−	+	+	+	−	−	−	−	−	−	−
	10	−		−	+	+	+	−	−	−	−	−	−	−
	19	−	−		+	+	+	−	−	−	−	−	−	−
a'b'	12	+	+	+		−	−	−	−	−	−	−	−	−
	14	+	+	+	−		−	−	−	−	−	−	−	−
	15	+	+	+	−	−		−	−	−	−	−	−	−
A'b'	21	−	−	−	−	−	−		−	−	−	−	+	+
	22	−	−	−	−	−	−	−		−	−	−	+	+
	23	−	−	−	−	−	−	−	−		−	−	+	+
	24	−	−	−	−	−	−	−	−	−		−	+	−
	26	−	−	−	−	−	−	−	−	−	−		+	+
a'B'	13	−	−	−	−	−	−	+	+	+	+	+		−
	20	−	−	−	−	−	−	+	+	+	−	+	−	

FIG. 7. *Table showing the results of pairing, in all possible combinations, a series of 13 monosporous cultures from a single fruit body of a European collection of Panus stypticus, No. 5122.*

		AB									ab						Ab		ab + aB
		1	2	3	6	11	12	13	14	19	21	4	5	7	8	10	15	16	17	18	20	22	9
AB	1		−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+
	2	−		−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−
	3	−	−		−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+
	6	−	−	−		−	−	−	−	−	−	+	+	+	+	−	−	+	−	−	−	−	+
	11	−	−	−	−		−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+
	12	−	−	−	−	−		−	−	−	−	+	−	−	−	−	+	+	−	−	−	−	+
	13	−	−	−	−	−	−		−	−	−	+	+	+	+	+	+	+	+	−	−	−	+
	14	−	−	−	−	−	−	−		−	−	+	+	+	+	+	+	+	+	−	−	−	+
	19	−	−	−	−	−	−	−	−		−	+	+	+	−	+	−	+	+	−	−	−	+
	21	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	−	−	−	+
ab	4	+	+	+	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−
	5	+	+	−	+	+	−	+	+	+	+	−		−	−	−	−	−	−	−	−	−	−
	7	+	+	−	+	+	−	+	+	+	+	−	−		−	−	−	−	−	−	−	−	−
	8	+	+	−	+	+	−	+	+	−	+	−	−	−		−	−	−	−	−	−	−	−
	10	+	+	−	−	+	−	+	+	+	+	−	−	−	−		−	−	−	−	−	−	−
	15	+	+	−	−	+	+	+	+	−	+	−	−	−	−	−		−	−	−	−	−	−
	16	+	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−
	17	+	+	−	−	+	−	+	+	+	+	−	−	−	−	−	−	−		−	−	−	−
Ab	18	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	+
	20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	+
	22	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		+
ab+aB	9	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	

FIG. 8. *Table showing the results of pairing, in all possible combinations, a series of 22 monosporous cultures from a single fruit body of an American collection of Panus stypticus, No. 5105.*

of Kniep (30), represent the pairs of allelomorphic factors that are assumed to control the pairing reactions of the monosporous mycelia in tetrapolar forms. The table for the series of pairings of collection No. 5105 is given because this series and that for collection No. 5122 are the only two of eight made that did not show complete fertility between monosporous cultures of compatible groups. This is a repetition of similar instances of incomplete incompatibility found by other workers in other species.

In Fig. 8 it may be seen that culture No. 5105–9 has, with one exception, paired with all the monosporous cultures of the two groups having the interfertility factors *AB* and *Ab* respectively. It has been assumed that two spores were picked up together when the culture was isolated and that it is composed of the haploid mycelia *ab* and *aB* which, being incompatible, have produced no clamp connections until paired with the compatible mycelia *AB* and *Ab*. The explanation, however, may be that there has been a mutation of the *B* factor in this mycelium and that the mycelium now has the interfertility factors *aB'*. No attempt was made to isolate the diploid mycelium from these pairings. There is also the possibility, although the writer has not seen such basidia in fruit bodies of *P. stypticus*, that the spore from which culture No. 5105–9 originated may have been produced on a three-spored basidium and have received two incompatible nuclei from the four nuclei present in the basidium after reduction division.

In addition to series of pairings between monosporous mycelia isolated from single sporophores, pairings were made between monosporous mycelia from different collections, i.e. between American and American collections, between European and European collections, and between American and European collections. A list of these pairings with their results is given in Table III. The results are presented graphically in Fig. 9 where the sign in each square represents the results of a series of pairings between mono-

		NORTH AMERICAN (LUMINOUS)					EUROPEAN (NON-LUMINOUS)				
		1532	2190	2191	5105	6004	1667	2118	2188	5120	5122
NORTH AMERICAN (LUMINOUS)	1532		+	±	+	+	+	+			
	2190	+					+	+			
	2191	±			+	±					
	5105	+		+		+				+	
	6004	+		±	+						+
EUROPEAN (NON-LUMINOUS)	1667	+	+					+	+	+	+
	2118	+	+				+		+	+	+
	2188						+	+		+	+
	5120				+		+	+	+		+
	5122					+	+	+	+	+	

FIG. 9. *Table showing the results of pairing, in all possible combinations, series of monosporous mycelia from different collections of the American and European forms of Panus stypticus.*

TABLE III

THE RESULTS OF SERIES OF PAIRINGS BETWEEN MONOSPOROUS CULTURES FROM DIFFERENT COLLECTIONS OF *Panus stypticus*

Culture number	Age when paired	Paired with culture number	Age when paired	Number of pairings	Clamp connections	
					Present	Absent
American×*American*						
1532	1 yr. 147 days	2190	160 days	1	1	0
	5 yr. 280 days	2191	4 yr. 293 days	12	11	1
	5 yr. 282 days	5105	2 yr. 23 days	12	12	0
	5 yr. 280 days	6004	1 yr. 63 days	12	12	0
2191	4 yr. 295 days	5105	2 yr. 23 days	16	16	0
	4 yr. 293 days	6004	1 yr. 63 days	16	14	2
5105	2 yr. 20 days	6004	1 yr. 62 days	16	16	0
European×*European*						
1667B	5 yr. 198 days	2118A	5 yr. 21 days	4	4	0
	5 yr. 198 days	2118B	5 yr. 21 days	12	12	0
	5 yr. 198 days	2188A	4 yr. 326 days	12	12	0
	5 yr. 198 days	5120	2 yr. 38 days	8	8	0
	5 yr. 198 days	5122	2 yr. 38 days	16	16	0
2118B	5 yr. 83 days	2188A	5 yr. 5 days	12	12	0
	5 yr. 83 days	2188B	5 yr. 5 days	4	4	0
	5 yr. 83 days	5120	2 yr. 102 days	4	4	0
	5 yr. 83 days	5122	2 yr. 102 days	16	16	0
2188A	5 yr. 5 days	5120	2 yr. 102 days	3	3	0
	5 yr. 5 days	5122	2 yr. 102 days	12	12	0
2188B	5 yr. 5 days	5120	2 yr. 102 days	1	1	0
	5 yr. 5 days	5122	2 yr. 102 days	4	4	0
5120	2 yr. 102 days	5122	2 yr. 102 days	4	4	0
American×*European*						
1532	55 days	1667A	About 185 days	16	16	0
	1 yr. 303 days	1667B	1 yr. 237 days	1	1	0
	1 yr. 170 days	2118B	229 days	1	1	0
2190	162 days	1667B	1 yr. 75 days	1	1	0
	160 days	2118B	229 days	1	1	0
5105B	125 days	5120	118 days	56	56	0
6004	333 days	5122	1 yr. 285 days	64	64	0

sporous mycelia of two different collections. The signs used are as follows:

(+) to indicate that clamp connections have been found in every pairing of the series.

(+.) to indicate that clamp connections have been found in every pairing of the series but one.

(±) to indicate that clamp connections have been found in the majority of the pairings of the series.

Where no sign appears in the square, the series of pairings has not been made.

The results of 336 pairings have shown that, with three exceptions, complete fertility exists between the monosporous mycelia of all the collections paired, whatever their origin or host. The three exceptions are to be found in two series of pairings between monosporous mycelia of the American collections, namely 2191 × 6004, where out of a series of 16 pairings two failed to produce clamp connections, and 1532 × 2191 where in one pairing out of 12 no clamp connections were formed.

Inheritance of Luminosity

When it was found that monosporous mycelia of the luminous and non-luminous forms of *P. stypticus* were interfertile, an investigation into the problem suggested by Buller (6) as to whether or not luminosity in this fungus is an inherited character and, if inherited, the manner in which inheritance occurs was undertaken.

As a preliminary step, the diploid mycelium produced by the union of monosporous mycelia of the luminous American and the non-luminous European forms was recovered from the pairings listed below and examined for luminosity. The diploid mycelium from every pairing was found to be luminous.

Luminous		*Non-luminous*	*Luminous*		*Non-luminous*
1532–14	×	1667B–7	5105B–6	×	5120–9
1532–14	×	2118B–4	5105B–8	×	5120–9
2190–3	×	1667B–7	5105B–8	×	5120–20
2190–3	×	2118B–4	5105B–16	×	5120–20
5105B–1	×	5120–1	5105B–18	×	5120–12

Subsequently, two monosporous cultures were selected from each of the interfertility groups formed by the monosporous mycelia of the luminous American collection No. 6004 and the non-luminous European collection No. 5122 (Figs. 6 and 7). The eight monosporous mycelia of the luminous form were paired in all possible combinations with the eight monosporous mycelia of the non-luminous form (Fig. 10). Clamp connections were found

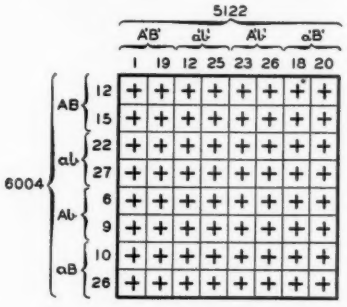
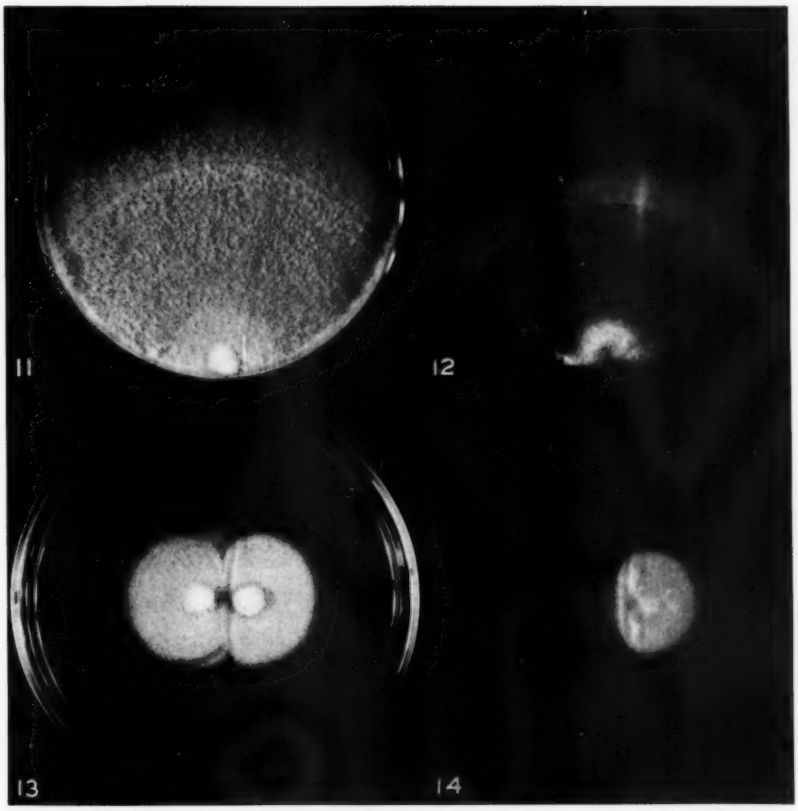


FIG. 10. *Table showing the results of pairing, in all possible combinations, eight monosporous mycelia from a single fruit body of the luminous American collection of Panus stypticus, No. 6004, with eight monosporous mycelia from a single fruit body of the non-luminous European collection, No. 5122.*



Panus stypticus cultures grown on 1.25% malt agar. ×¾ approximately. FIG. 11. *Diploid mycelium, four weeks old, from a pairing between a haplont, No. 6004–6, of the luminous American form and another haplont, No. 5122–12, of the non-luminous European form, photographed by reflected light.* FIG. 12. *The same culture as Fig. 11 photographed by its own light.* FIG. 13. *A two-weeks–old pairing between a non-luminous haplont, No. 5122–20, on the left, and a luminous haplont, No. 6004–6, on the right, photographed by reflected light.* FIG. 14. *The same pairing as Fig. 13 photographed by its own light. Note the luminosity of the haplont, No. 6004–6, and of the diploid mycelium formed by the union of the two haplonts in the area between the two inocula.*

PLATE IV

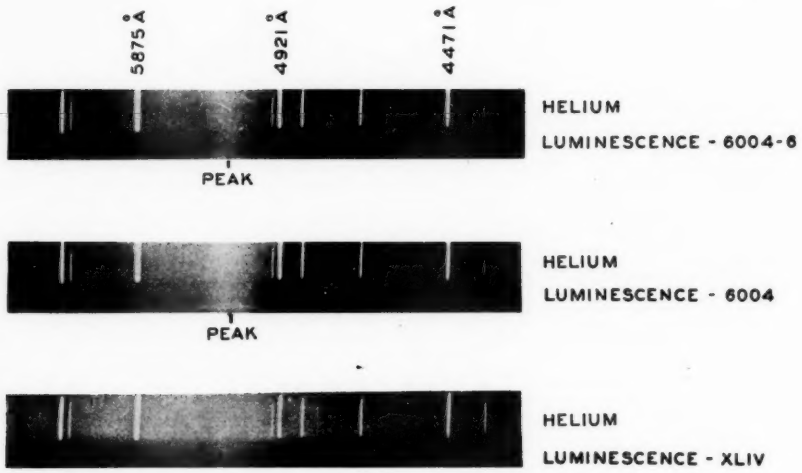


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FIG. 15. *Spectra emitted by three cultures of Panus stypticus with helium comparison spectra. 6004–6: a monosporous culture and 6004: a polysporous culture of the luminous form of North American origin, XLIV: a diploid culture derived from the pairing of monosporous cultures of the luminous American form and the non-luminous European form, 6004–6 × 5122–20.*

in each of the 64 pairings and the diploid mycelium isolated from all the pairings was found to be luminous.

Photographs of one of these pairings, 6004–6 × 5120–20, taken by reflected light and in the dark by its own light are given in Plate III. The luminosity of the diploid mycelium extending from the line of union between the two haplonts to the inoculum of the non-luminous haplont is clearly shown in the photograph taken in the dark.

The diploid mycelium produced by the pairings was isolated by transferring a very small piece of the mycelium from between the two inocula to a fresh malt agar slant and transferring this culture again several times. In 13 pairings, however, in order to avoid any possibility of the haploid mycelium being carried over, a minute inoculum from the diploid culture was placed on a malt agar plate and, as soon as a few hyphae had grown down into the agar, the inoculum was removed and a single hypha in the agar below was cut out with a fine needle, under the low power of a compound microscope, and transferred to a test-tube of malt agar. Isolation of single hyphae from the margin of a culture was found to be impossible as the individual hyphae there grew very close together.

Three of the 13 cultures, each derived from a single diploid hypha, namely XXXIX (6004–6 × 5122–12), XLIV (6004–6 × 5122–20), and XLV (6004–9 × 5122–1), were grown on sections of *Betula papyrifera* branches, 6.5 to 9 cm. in diameter and 16 to 30 cm. long, which had the bark and part of the wood removed from one side. The sections were placed on sphagnum moss in a wide-mouthed jar, a little water added, the mouth of the jar covered with absorbent cotton, and the whole sterilized at 15 lb. steam pressure for one-half hour. The cultures were kept for five months in diffuse light in the laboratory and then from November until April were placed open under a bell jar in diffuse light in a greenhouse.

In March, 10 months after inoculation, two of the cultures, Nos. XXXIX and XLIV, had produced numerous rudimentary fruit bodies. Three of the fruit bodies of culture No. XLIV matured and shed spores (Plate I). When examined in the dark the gills were found to be brightly luminous. Five hundred and seven germinating spores from one of the three hybrid fruit bodies were isolated. Of these sporelings, 365 continued to grow and were examined for luminosity 27 and 54 days after isolation. Of the 365 cultures 198 were found to be luminous and 187 non-luminous, a ratio roughly of 1 : 1.

Nine of the luminous and 11 of the non-luminous monosporous cultures from the hybrid fruit bodies were paired together in all possible combinations with the results given in the table in Fig. 16. In this table the non-luminous cultures are marked by a line under the number of the culture. The results of the pairings show that the series of cultures is tetrapolar with both luminous and non-luminous cultures in each of the four interfertility groups.

It has been found (Hanna (19)) that the interfertility factors of the monosporous mycelia from a first generation fruit body may be determined by

		Ab						a'B'						ab						AB'	
		1	4	11	20	22	24	7	14	16	17	18	21	2	8	10	12	13	15	3	19
Ab	1		−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	4	−		−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	11	−	−		−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	20	−	−	−		−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	22	−	−	−	−		−	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	24	−	−	−	+	−		+	+	+	+	+	+	−	−	−	−	−	−	−	−
a'B'	7	+	+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−	−	−
	14	+	+	+	+	+	+	−		−	−	−	−	−	−	−	−	−	−	−	−
	16	+	+	+	+	+	+	−	−		−	−	−	−	−	−	−	−	−	−	−
	17	+	+	+	+	+	+	−	−	−		−	−	−	−	−	−	−	−	−	−
	18	+	+	+	+	+	+	−	−	−	−		−	−	−	−	−	−	−	−	−
	21	+	+	+	+	+	+	−	−	−	−	−		−	−	−	−	−	−	−	−
ab	2	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	+	+
	8	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	+	+
	10	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	+	+
	12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	+	+
	13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	+	+
	15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		+	+
AB'	3	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+		−
	19	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	

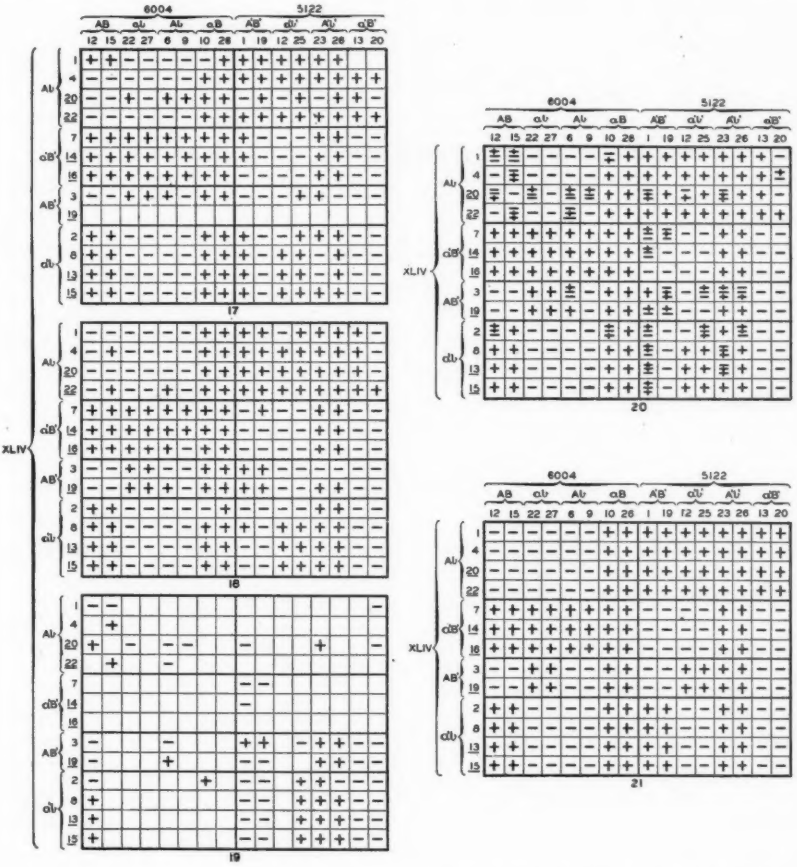
FIG. 16. *Table showing the results of pairing, in all possible combinations, a series of 20 monosporous mycelia from a single hybrid fruit body, No. XLIV, produced by the diploid mycelium from a pairing between a luminous and a non-luminous monosporous mycelium of Panus stypticus (6004–6 × 5122–20).*

backcrossing the F_1 mycelia with the mycelia of all four interfertility groups of both parents, since the interfertility factors of the parent mycelia are known and the monosporous mycelia from the F_1 fruit body pair only with those parent mycelia with which they have no common factor. In order, therefore, to determine the interfertility factors that should be assigned to each of the four groups of XLIV monosporous mycelia shown in Fig. 16, mycelia from each of the four groups were paired with each of the four groups of both parents. The results of these pairings are given in Fig. 17 where the interfertility factors for the monosporous mycelia of the parent collection, No. 6004, are designated as AB, ab, Ab, and aB and for the monosporous mycelia of the other parent collection, No. 5122, as $A'B'$, $a'b'$, $A'b'$, and $a'B'$.

Since the two monosporous mycelia which gave rise to the hybrid fruit body, No. XLIV, had the interfertility factors Ab and $a'B'$ (6004–6 × 5122–20), the monosporous mycelia isolated from this fruit body would, if reduction division took place in the usual way, be expected to have the factors Ab, $a'B'$, AB', and $a'b$, and to be compatible with the parent mycelia as follows:

Ab	mycelia with	aB, $A'B'$, $a'b'$, $A'b'$, $a'B'$	mycelia
$a'B'$	mycelia with AB, ab, Ab,	aB, $A'b'$	mycelia
AB'	mycelia with	ab, aB, $a'b'$, $A'b'$	mycelia
$a'b$	mycelia with AB,	aB, $A'B'$, $A'b'$	mycelia

In the series of pairings shown in Fig. 17, however, not all the mycelia of one group of monosporous mycelia from the hybrid fruit body reacted in the same way when paired with the parent mycelia, and it was not clear from the pairings which of the pairs of factors should be assigned to the different groups. All of the pairings, therefore, were repeated and many were made a third



FIGS. 17 TO 21. *Panus stypticus.* FIGS. 17 TO 19. *Tables showing the results of pairing 13 monosporous mycelia of the hybrid fruit body, No. XLIV, with two monosporous mycelia from each of the four groups of both parents.* FIG. 20. *A composite table of the results given in Figs. 17 to 19.* FIG. 21. *Ideal picture of the results expected when the actual results of the pairings are taken into consideration.*

time. The results of these additional pairings are shown in Figs. 18 and 19. In Fig. 20 a composite table is given of all three series of pairings.

The results of these series of pairings indicate that monosporous mycelia Nos. 1, 4, 20, and 22 of the fruit body XLIV have the interfertility factors *Ab*, since, with the exception of two pairings, they are compatible with the parent mycelia having the factors *aB*, *A'B'*, *a'b'*, and *a'B'*, although some fertile pairings have been found between these mycelia and monosporous mycelia of each of the other groups. Likewise XLIV monosporous mycelia Nos. 7, 14, and 16 appear to have the interfertility factors *a'B'*, although three fertile pairings have occurred between two of these mycelia and parent mycelia bearing the factors *A'B'*.

The remaining two groups of monosporous mycelia, Nos. 3 and 19 and Nos. 2, 8, 13, and 15, both of which had one factor from each parent (*AB'* and *a'b*), when paired with the No. 6004 monosporous mycelia, appear to have the factors *AB'* and *a'b* respectively, although XLIV–3 and XLIV–19 with the factors *AB'* have paired with 6004–6 with the factors *Ab*. These same two groups of mycelia, if assigned the factors *AB'* and *a'b* respectively as suggested by their reactions with the No. 6004 monospores, show many compatible pairings between mycelia with a common interfertility factor and incompatible pairings between mycelia where no common factor is present when they are paired with the No. 5122 monosporous mycelia, and would seem to have the factors *a'b* and *AB'* respectively rather than the factors *AB'* and *a'b*. As, however, the pairings of these monospores with the No. 6004 monospores gave clearer results, these two groups of F_2 monosporous mycelia have been assigned the factors *AB'* and *a'b* respectively in Figs. 16 to 20.

In the whole series many compatible pairings have taken place between monosporous mycelia that have a common interfertility factor. They have occurred usually once or sometimes twice in a succession of three pairings of the same two mycelia, showing an instability or weakness of reaction not present in the majority of the compatible pairings in the series where no common factor was present. The clamp connections formed in these pairings were true not false clamp connections as Quintanilha (44) and Biggs (4) have found in illegitimate pairings in some species.

Quintanilha (43) and others have found that "illegitimate" pairings such as these, that is fertile pairings between haploid mycelia having a common interfertility factor, occur more frequently in some tetrapolar species between haploid mycelia that have one pair of factors in common rather than the other pair, that is when the *B* or *b* factors rather than the *A* or *a* factors are alike or vice versa depending on which factors are assigned to the group. In the series of pairings described here, the illegitimate pairings are not confined to mycelia that have one pair of factors in common but have occurred when any one of the four interfertility factors *A*, *b*, *a'*, or *B'*, have been held in common:

Illegitimate pairing	Common factor	Illegitimate pairings	
		No.	%
$Ab \times AB$	A	5	62.5
$Ab \times ab$	b	1	12.5
$Ab \times Ab$	A and b	3	37.5
$aB' \times A'B'$	B'	3	50
$AB \times Ab$	A	2	50
$AB' \times A'B'$	B'	4	100
$a'b \times a'b'$	a'	7	87.5

In three of the pairings both interfertility factors, $Ab \times Ab$, were held in common by the paired mycelia. It may be noted also that when the haploid mycelia of the hybrid fruit body XLIV were paired together in all possible combinations, Fig. 16, the haploid mycelia XLIV–20 × XLIV–24, having these same two factors, $Ab \times Ab$, also paired with each other.

Quintanilha (43) found that illegitimate pairings in *Coprinus fimentarius* occurred more frequently when the mycelia paired were young and often disappeared with the ageing of the mycelia. In this series, however, the mycelia were fully mature: 6004 haplonts—2 yr., 338 days; 5122 haplonts—3 yr., 228 days; XLIV haplonts—169 days.

It has been suggested by Kniep (31) that the ability of two monosporous mycelia to pair is dependent upon certain quantitative differences in the fertility factors, differences that have a maximum and a minimum beyond which pairing does not take place. When, for example, in certain individuals the difference between the B factors is sufficiently great, B (or b) individuals can pair with other B (or b) individuals if the equality of the A factors does not stand in the way. If, on the other hand, a difference approaching a minimum occurs, a slight variation means that the minimum difference for copulation is not reached and sterility results.

Whether the irregularity in the behaviour of the monosporous mycelia of the hybrid fruit body XLIV when paired with the mycelia of the parent generation is the result of quantitative changes in the factors such as this or whether it is brought about by mutations or other chromosome changes has not been determined.

Discussion

Since the diploid mycelium and hybrid fruit bodies from a cross between the luminous and non-luminous forms of *P. stypticus* are luminous and the haploid mycelia derived from such a cross may be separated into two approximately equal luminous and non-luminous groups, it must be concluded that the factor for luminosity in this fungus is an inherited one and that luminosity is dominant over non-luminosity. Since, also, both luminous and non-luminous mycelia are to be found in all four interfertility groups of haploid mycelia of the F_1 hybrid fruit body, it is evident that the factor for luminosity and its allelomorph form all possible combinations with the interfertility factors.

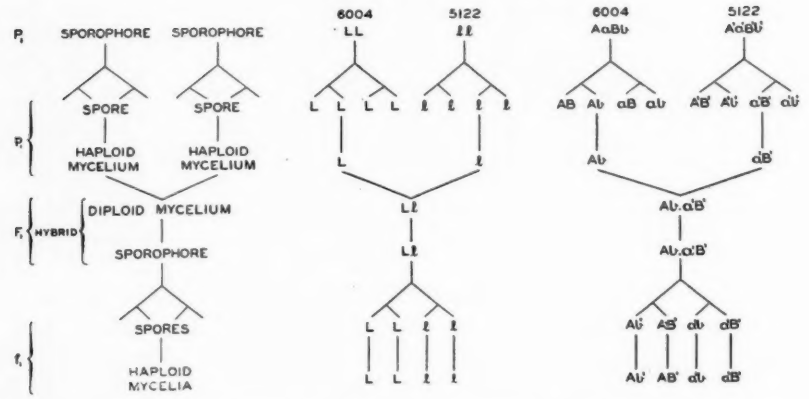


FIG. 22. *Diagrams showing the inheritance in Panus stypticus of the luminosity and interfertility factors in a cross between haploid mycelia of a luminous American collection, No. 6004, and a non-luminous European collection, No. 5122.*

The method of inheritance of the luminosity factor, suggested by these experiments, is presented graphically in Fig. 22 where the factor for luminosity is represented by the letter *L* and its allelomorph, non-luminosity, by the letter *l*. The sporophore of the luminous American form or P_1 generation possesses the factors *LL* and the sporophore of the non-luminous European form, their allelomorphs *ll*. After reduction division of the fusion nucleus in the basidia of the P_1 generation, each of the spores and haploid mycelia of the luminous sporophore carries one factor for luminosity, *L*, and each of the spores and haploid mycelia of the non-luminous sporophore its allelomorph *l*. The diploid mycelium resulting from the union of luminous and non-luminous haploid mycelia and the sporophores produced by such diploid mycelia possess the two factors *Ll*. Upon reduction division the luminosity factors are divided equally among the four spores and the ratio of luminous to non-luminous haploid mycelia in the f_1 generation is 1 : 1, which is the Mendelian ratio of a dominant to its allelomorph in the gametes of the f_1 generation.[1]

The gametophytic and sporophytic generations characteristic of the life history of most plants are distinguished from each other by the number of chromosomes present in the nuclei of each generation, the former having the single or haploid number of chromosomes and the latter the double or diploid number. In the heterothallic Basidiomycetes the basidiospores and the monosporous mycelia arising from them, having nuclei with the haploid number of chromosomes, constitute the haploid or gametophytic phase in the life history of these fungi. When two compatible haploid mycelia unite, a diploid mycelium is formed in which the haploid nuclei are associated in pairs within the cells. The paired nuclei divide conjugately during the devel-

[1] *In assigning symbols to the different generations here and in Fig. 22 the scheme followed by Allen (1, 2) is used, namely* P_1, F_1, F_2 *etc. for the diploid generations and* p_1, f_1, f_2 *etc. for the haploid generations.*

opment of the mycelium and fruit bodies until in the basidium they unite to form a single fusion nucleus. The binucleate mycelium and fruit bodies and the basidia constitute the diploid or sporophytic phase.

The alternation of haploid and diploid phases in the life history of the Basidiomycetes may be compared to the alternation of gametophytic and sporophytic generations in the higher plants, with this difference: in the higher plants the nuclei of the gametes fuse when they come together within a single cell whereas in the heterothallic Basidiomycetes the two haploid nuclei remain associated together without union throughout an appreciable stage of the life cycle. Their fusion in the basidium may be considered a delayed sexual fusion.

It has been shown by the work of Zattler, Gilmore, Dickson, Nobles, and Quintanilha and Balle already reviewed, and by the study of the inheritance of uredospore colour, pustule type, and pathogenicity in the Uredinales and the inheritance of the general morphology and consistency of the sori, the colour of the peridia of the sori, the degree to which the host plant may be stunted, and pathogenicity in the Ustilaginales that the genes governing the inheritance of different characters behave in the dikaryotic mycelium and fruit bodies of the Basidiomycetes, although contained within two separate nuclei, as they do in higher plants where they have come together within a single nuclear membrane.

Dodge (14) writes that a cell may be considered diploid only when its nucleus contains the double number of chromosomes and hence cells containing two haploid nuclei, even though of opposite "sex", are not diploid but rather dikaryotic haploid partnerships and that the true diploid in the Basidiomycetes is to be found only in the basidium where the nucleus, after fusion has taken place, contains the double number of chromosomes. In dealing with the genetics of fungi possessing such dikaryons he considers the dikaryotic mycelium resulting from the union of the two haploid mycelia of different genetic origin not as true hybrids but rather as graft hybrids or chimaeras and that since only diploid nuclei can be heterozygous any apparent dominance in such mycelia is a false one.

Johnson and Newton (25), Zattler (55), Stakman, Levine, and Cotter (49), and Buller (7, 8) have expressed the opposite view that since the two sets of genes within a diploid cell have been shown to act as though contained within a single nuclear membrane the dikaryotic mycelium and fruit bodies are truly diploid and are true hybrids.

The evidence of dominance of the factor for luminosity in the diploid mycelium and fruit bodies produced by pairing luminous and non-luminous haploid mycelia of *Panus stypticus* adds further evidence in support of the latter authors' views.

Acknowledgments

These investigations were carried out at the Department of Botany, University of Toronto, and at the Division of Botany and Plant Pathology, Department of Agriculture, Ottawa. The writer wishes to express her

appreciation and gratitude to Professor H. S. Jackson for his advice and criticism during the course of the work, and to thank Dr. H. T. Güssow, Dominion Botanist, for his unfailing interest and for supplying the European material, Dr. A. H. R. Buller for his interest in the problem which he suggested in his Researches on Fungi, Volume III, and for supplying three of the American collections, Dr. J. W. MacArthur for reading and criticizing the manuscript, and Dr. Irene Mounce for her helpful suggestions. She wishes also to thank Dr. L. E. Howlett of the Division of Physics and Engineering of the National Research Council of Canada for his kindness in taking the photographs of the spectra of the cultures.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. AUGUST, 1942 NUMBER 8

POLARIZATION AND PROGRESSION IN PAIRING

II. PREMEIOTIC ORIENTATION AND THE INITIATION OF PAIRING[1]

BY STANLEY G. SMITH[2]

Abstract

Homologous chromosomes in the Diptera associate side by side in pairs at each and every anaphase (somatic pairing) and reappear in the following prophases relationally coiled. In plants and animals other than Diptera the homologues at anaphase (with one exception) show no such specific attraction: at prophase the relational coiling of homologues is here supplanted by a relational coiling of sister chromatids. The one exception arises at the anaphase of the last premeiotic division—homologues become associated in pairs and reappear in the following prophase relationally coiled.

In the Diptera the chromosomes are single at each and every anaphase: in other animals and plants the chromosomes are double at all anaphases except that of the last premeiotic division. Hence at this latter division the attraction in pairs between chromatids is replaced by an attraction between pairs of homologues.

That somatic pairing is characteristic of the Diptera has been known since the pioneer work of Stevens (67) and Metz (39). Stevens stated with regard to nine species that "In many cases the prophases of spermatogonia and first spermatocytes resemble each other very closely, the members of each pair being twisted together in both.", and pointed out that "In the spermatocyte we get complete synapsis and reduction; in the spermatogonium only a foreshadowing of reduction . . .". Metz found association of homologues at metaphase and anaphase of diploid mitoses in about 80 species, from the lowest to the highest of the order. Metz and Nonidez (41) working on *Asilus sericeus* noted that in spermatogonial prophase ". . . each aggregate gives rise to a long double thread by a process of attenuation or uncoiling (fig. 1)." At the final spermatogonial anaphase the chromosomes go to the poles associated in pairs, and the late anaphase crowding makes pairing more intimate. The crowding is relaxed at telophase and the pairs are seen to be intimately associated, so much so, ". . . that the duality is often obscured." The resting stage is very brief, no leptotene was observed, and no doubleness was visible, except as paired chromosomes. In *Asilus notatus* similar conditions were found to hold, and the authors were led to state ". . . it may be possible to bring all types [of Diptera] within a common general scheme in which synapsis occurs in the anaphase or telophase of the final spermatogonial division." (42).

[1] *Manuscript received May 23, 1942.*

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A study now in progress of two dipterous parasites, *Bessa selecta* and *Phorocera hamata* (?), has shown these same general features in somatic mitoses. The pairs of homologous chromosomes are associated closely at mitotic prophase by relational coiling (*contra* Darlington (12, p. 235)); as far as can be seen the individual chromosomes comprising each pair are single until late prophase when they become uncoiled, visibly double, and associated only at the centromeres. Visible division of the centromeres is followed by separation of the chromatids of the paired chromosomes, those of one moving to opposite poles each closely paralleled by a chromatid from the other chromosome. By telophase these pairs are intimately associated side by side and reappear in the next prophase again relationally coiled.

Within the last two years four papers have appeared which indicate that the chromosomes in some liliaceous plants are polarized at the prophase of meiosis (6, 13, 21, and 65) as has been demonstrated by Belar (7) in *Tradescantia*. Direct demonstration of polarization is difficult in most Liliaceae, presumably on account of the great length of the chromosomes at early prophase. Darlington (12, p. 91) originally denied the occurrence of polarization in ". . . some Liliaceae except merely as a relic of the orientation of the chromosomes, with their centromeres towards the pole, at the preceding telophase . . ." Apparently he considered polarization to be an active process of orientation during meiotic prophase, and, moreover, still does so (13). The occurrence of this "active" polarization is questionable–Gelei (23, 24) to the contrary; in any case, the evidence of immobility during the resting stage points to a polarization resulting from the last premeiotic anaphase separation (designated by Gelei (23, 24), Rabl's (58) orientation); the centromeres are grouped together against the nuclear membrane and the distal ends are concomitantly grouped at the opposite side of the cell (65). Such an orderly arrangement appears to be a prerequisite to orderly pairing. It is presumed that following spatial derangement the bivalents become interlocked (13, 65). Interlocking, however, is tolerably rare, certainly rarer than could possibly be expected unless the position of homologues were predetermined, or unless pairing were initiated at one point only, the centromere, and progressed outwards from that point. Therefore the question is, are the positions assumed by pairing homologues predetermined? A consideration of the literature supplies a reasonable answer (Table I).

As a result of work on dipterous parasites, on sawflies, grasshoppers, and various plants, the writer has been led to the view that in the premeiotic telophase there is an active mutual selection of maternal and paternal homologues during their movement towards the poles. This concept recalls to mind the statement of Wilson (76) regarding somatic pairing:— "It seems quite possible that the way for synapsis may be prepared already in a very early pre-synaptic stage, by a definite regrouping of the chromosomes that may take place before the leptotene loops are formed as such." Supporting evidence for this thesis comes largely from the literature, although it has been pointed out earlier (64) that there is no characteristic leptotene at oogenesis

TABLE I

OCCURRENCE OF PREMEIOTIC ASSOCIATION OF HOMOLOGOUS CHROMOSOMES

(i) Prophase of second cleavage	Arthropoda, Insecta (Diptera)	*Drosophila melanogaster*	Huettner (28)
(ii) All embryonic nuclei	Arthropoda, Insecta (Diptera)	*Drosophila "ampelophila"*	Stevens (67)
		Many species	Metz (39)
(iii) Probably all somatic nuclei	Arthropoda, Insecta (Diptera)	*Culex pipiens*	Taylor (73); Hance (26)
		Ca. 80 species	Metz (39)
(iv) All gonial metaphases and anaphases	Arthropoda, Insecta (Hymenoptera)	*Apis mellifica*	Nachtsheim (51)
	(Diptera)	Various species	Stevens (67, 69); Metz (39)
		Culex pipiens	Whiting (75)
	Rotifera	*Asplanchna intermedia*	Tauson (72)
(v) Several gonial metaphases	Annelida, Polychaeta (Errantia)	*Ophryotrocha puerilis*	Dehorne (14)
	Arthropoda, Insecta (Orthoptera)	*Syrbula*	Montgomery (47)
		Various species	Eisentraut (18)
	(Hemiptera)	Various species	Montgomery (48)
	Vertebrata, Amphibia (Anura)	*Alytes obstetricus*	Janssens and Willems (32)
	(Urodela)	*Plethedon*	Montgomery (46)
		Salamandra	Dehorne (15)
(vi) Last (?) gonial metaphase	Arthropoda, Insecta (Hemiptera)	Three genera	Slack (63)
	(Hymenoptera)	*Pteronidea ribesii*	Peacock and Sanderson (56); Sanderson (60)
	Mollusca, Gasteropoda (Pulmonata)	*Helix pomatia*	Ancel (1)
	Vertebrata, Marsupiala	Five genera	Koller (33)
(vii) Last gonial anaphase–telophase	Coelenterata, Cnidaria (Hydrida)	*Hydra*	Downing (16)
	Platyhelminthes, Turbellaria	*Dendrocoelum lacteum*	Gelei (22); (retracted (23))
	Annelida, Chaetopoda (Oligochaeta)	*Allolobophora foetida*	Foot and Strobell (20)
	Arthropoda, Onychophora	*Peripatus balfouri*	Montgomery (44)
	Crustacea	Various species	Nichols (53, 54)
	Myriapoda (Chilopoda)	*Scolopendra*	Blackman (9)
	Insecta (Orthoptera)	Various species	McClung (35, 36)
		Dissosteira carolina	Nebel and Ruttle (52)
		Brachystola magna	Sutton (71)
	(Dermaptera)	*Forficula auricularia*	Callan (10)*
	(Hemiptera)	Various species	Montgomery (45)
	(Coleoptera)	*Blaps lusitanica*	Nonidez (55)
	(Hymenoptera)	Three species	Smith (unpub.)
	(Diptera)	Various species with chiasmata	Stevens (67, 68); Moffett (43)
		Various species without chiasmata	Metz (40); Metz and Nonidez (41, 42)
	Arachnida (Araneida)	*Schizocosa crassipes*	Hard (27)
	Polyzoa, Endoprocta	*Pedicellina americana*	Dublin (17)
	Chaetognatha	*Sagitta bipunctata* ♂	Stevens (66)
(viii) After last gonial division but "before conjugation"	Platyhelminthes, Turbellaria	*Planaria lactea*	Arnold (3)
	Arthropoda, Insecta (Hemiptera)	*Euschistus variolarius*	Foot and Strobell (19)
		Corixa distincta	Prokofiewa (57)
	(Coleoptera)	*Hydrophilus piceus*	Arnold (2)
		Tenebrio molitor	Stocking (70)
	(Hymenoptera)	*Diprion polytomum*	Smith (64)
	Chaetognatha	*Sagitta bipunctata* ♀	Stevens (66)

* *Only the sex chromosomes were seen to pair at telophase, at which time they are positively heteropycnotic. It would be of interest to know whether the autosomes associate at the anaphase–telophase of this division.*

in the sawfly, *Diprion polytomum*; a statement later supported by the observation of apparent association of chromosomes in pairs in the last oogonial telophase (unpublished data).

That an association of homologues occurs at or by telophase of the last premeiotic division has been claimed by a number of authors (Table I), while the future assumption of such an association can be inferred from the observation of somatic pairing at metaphase by others. Regarding this, Stevens (66) states "These figures [spermatogonial divisions with the reduced number of chromosomes at telophase, see Fig. 19B] lead me to think that the so-called synapsis stage occurs in *Sagitta* at the close of the final spermatogonia division, the chromosomes uniting in pairs at the poles of the spindle." Of the bouquet stage in oogenesis she states "The regular arrangement of loops in such oocytes as are shown in Figs. 5 and 6, indicate the possibility that they may have begun their development after the last oogonia division without an intervening resting stage, and that the reduction in number to nine bivalent chromosomes may have recently taken place." She notes that she cannot be sure that a resting stage does not intervene between the union in pairs and the bouquet stage, where the nine loops ". . . have a somewhat crenate or beaded outline." (i.e., appear to be relationally coiled). In their later paper, which appears to have been completely overlooked in recent publications, Foot and Strobell (20) have unequivocal evidence for pairing at telophase in *Allolobophora foetida*. They state "Photo 1 shows an oögonial prophase in which 22 chromosomes can be clearly counted and Photo 2 is a young oöcyte in which 11 chromosomes can be counted with equal clearness. We have placed these Photos side by side in order to facilitate a comparison of the two groups of chromosomes. It would seem that such a comparison ought to throw some light on the method of pseudo-reduction, but it is evident this is not the case. The bivalent chromosomes of Photo 2 indicate merely that they have passed through some profound changes between the stages shown in Photos 1 and 2—changes which have presumably occurred after the telophase of the last oögonial division. We have, however, been unable to find any satisfactory evidence of the occurrence of these intervening stages. The strongest evidence we have that the bouquet stage is preceded by a rest stage, is the fact that a nucleolus . . . is frequently found in the bouquet nucleus." The reduced chromosomes are found at the extreme proximal end of the ovary on or near the periphery and ". . . in such close relationship to the oögonial metaphases that the indications are that they arise without the intervention of a typical resting stage." To the writer their Photo 2 represents the telophase of the last oogonial division. The polarized telophase chromosomes ". . . become longer, thinner and *more twisted* [present writer's italics] until finally they disappear and we have the typical resting oöcyte nucleus." All these stages occur in the ovariole. Then there follows a "leptotène, pachytène, and diplotène" in eggs liberated into the receptaculum ovorum ". . . and it is only exceptionally that the largest eggs at the extreme

distal end of the ovary have developed even to the leptotène stage". Hence there can be no doubt regarding the seriation of the stages.

The demonstration of telophase pairing in the Coelenterata, Platyhelminthes, Annelida, Arthropoda, Polyzoa, and Chaetognatha, etc., the occurrence of earlier conditions doubtless leading to it (Table I), and the observation of polarization in the majority of animals and many plants, taken together with deductive evidence for such an orientation in other plants, lead the writer to conclude that pairing in the premeiotic telophase is in some degree or other, the general rule for meiosis. But how could the telophase pairing be conditioned? Further consideration of the Diptera offers a tentative answer which is in agreement with numerous observations.

The homologous chromosomes of the Diptera associate in pairs at anaphase of mitosis whether in somatic, spermatogonial, or oogonial nuclei. The pairs in somatic prophase are relationally coiled and polarized ". . . suggesting the previous anaphase condition" (26). Somatic pairing is established in the egg before cleavage is completed (39), and according to Huettner (28) is visible for the first time at the prophase of the second cleavage division in *Drosophila melanogaster*. There is no attraction between the maternal and paternal homologues on the first cleavage metaphase plate, nor, in the fusion of the polar bodies, do the homologues, seen to be double as they emerge from the resting stage, show any regular attraction for one another, unless the usual arrangement of the three minute fourth chromosomes in the centre of the group be due to attraction. It appears probable (unpublished data) that the attraction between maternal and paternal homologues is initiated during the anaphase of the first cleavage. This, if true, is very significant for the paternal chromosomes at least have been double since the sperm resting stage (50), and have become single only at this anaphase. Hence, one is led to conclude that the late anaphase pairing (and all future somatic pairing) is conditioned by and compensates for the prior anaphase disjunction and consequent singleness of the chromatids.

Darlington and his co-workers consistently deny the doubleness of mitotic anaphase chromosomes (even though it is as consistently seen by numerous competent observers), maintaining that the doubleness arises during the resting stage. Observations to the contrary are ascribed to viewing a cylinder in optical longitudinal section. Huskins and Smith (31), among others, have demonstrated that in *Trillium erectum* the chromonemata are double while jointly helical—thus adequately meeting Darlington's criticism—so that bivalent chromosomes at metaphase are composed of eight threads. The leptotene chromosome is nevertheless single. To reconcile this apparent dualism Huskins (29) suggested that the splitting of the chromonema may be retarded in the last premeiotic division, but found that although this concept led to the resolution of certain difficulties, others then appeared.

Now if the Darlington school were correct in their contention that anaphase chromosomes are single and that attraction is effective only between pairs of single chromosomes, then the homologues should associate in pairs at each

anaphase and telophase[1], as they do in the Diptera (where the writer assumes they are single[2]), or else the pairing observed at pachytene would need be conditioned by some force other than attraction. The fact that somatic pairing does not ordinarily occur in animals other than Diptera, nor in plants, if such forms as *Spinacea* (25) are disregarded, is in agreement with the doubleness of their anaphase chromosomes.

Following Metz (40), Darlington (11) has concluded from an analysis of the conditions in *D. pseudo-obscura* that the association of the homologous autosomes at first meiotic metaphase is due to a specially exaggerated property of attraction in the Diptera. White (74), however, has described an anomalous type of meiosis in a mantid, *Callimantis antillarum*, in which, although chiasmata are absent, the chromosomes nevertheless remain paired. There is in this case no specially exaggerated property of attraction, for there is no somatic pairing either in spermatogonial *metaphases* or those of interstitial cells. White is led to state:— "It seems probable, therefore, that the chromosomes remain associated in bivalents right up to metaphase simply because the split (which normally appears at the end of pachytene) is, in *Callimantis*, delayed until later. Possibly . . . a 'pellicle' holds the chromosomes together."

If the alternative view is accepted, that the chromosomes are invariably double at anaphase, the conditions of singleness at leptotene, observed by such workers as Belar, Belling, and Newton, would demand a healing of the premeiotic split. Duality of the last premeiotic *telophase* chromosomes has been reported in Orthoptera by McClung (36), Robertson (59), and Nebel and Ruttle (52), while duality at the last premeiotic *anaphase* has been figured by Atwood (4) in *Gaillardia aristata* (Compositae). This author illustrates the doubleness at all stages from late metaphase of the last premeiotic division through the resting stage up to zygotene, but, because at no stage are the chromomeres resolved and because at no stage are all the chromosomes countable, the evidence is considered to be unconvincing. McClung's illustrations of Orthoptera chromosomes (and to a lesser extent those of Nebel and Ruttle) appear to establish both duality and telophase pairing, although McClung says of his own material:— ". . . these are not entirely satisfactory or convincing." Of more importance is the fact that no evidence is submitted that the doubleness is present before telophase, i.e., at the time of the establishment of pairing. In any case the whole significance of these observations hinges upon a determination of the positions of the centromeres and the positions of the chiasmata in relation to them (cf. 37).

[1] *That it is not the fact that the anaphase chromosomes are somatically coiled that is responsible for this failure of anaphase pairing follows from Barber's (5) contention that the attraction due to ". . . radial symmetry . . . will not be satisfied by union in twos but, as in the diplochromosomes, may accommodate more."*

[2] *The apparently contradictory evidence of doubleness found by Dr. B. P. Kaufmann ("Somatic Mitoses of Drosophila melanogaster." J. Morphol. 56 : 125-155. 1934.), is here reconciled by the assumption that the "giant cells" are tetraploid. Note the difference in type of "split" and in degree of somatic pairing in the giant and smaller cells of the ganglia.*

Belling (8) states that he does not regard as proved Darlington's conclusion that "Meiosis differs from mitosis in the nucleus entering prophase before the chromosomes divide instead of after they divide." The observation of leptotene doubleness is in agreement with the conditions observed in Orthoptera. Observations of leptotene singleness in other organisms are, the writer believes, not incompatible if the degree of telophase pairing is variable and somewhat inversely correlated with chromosome length (cf. 30). In no case at leptotene should the whole length of the chromosome be normally unpaired; the shorter the chromosome the more complete the pairing. The observation of pairing at preleptotene stages may in this way serve to harmonize some of the conflicting interpretations regarding chromosome structure at leptotene (see especially 20).

These considerations lead to the following conclusions:— (1) the meiotic pairing consummated at pachytene is initiated at the latest by the telophase of the last premeiotic division; (2) this is conditioned by the total (though in some cases only temporary) suppression of the split in the last division; following the disjunction of single chromatids in the last anaphase there is a compensating union of daughter homologous chromatids. Table I makes it clear that the regrouping of chromosomes preparatory to the association of homologues at the last premeiotic telophase may in some organisms (Sections iv, v, and vi) be a gradual process. Presumably the division of the chromosomes *per se* may be incompletely co-ordinated with the division of the cell. When the rate of division of the cell is rapid (as it is in the gonia), that of the chromosomes lags behind and allows partial attraction of homologues; this being necessarily cumulative, somatic pairing in various degrees may become established before the final telophase*.

The most that can be said for these considerations is that they may constitute general rules; exceptions are apparent, for example among coccids, other Hemiptera (61, 62), and Hepaticae (34).

Telophase pairing considered in relation to variability in the time of splitting and assumption of the relational coil has an obvious bearing on such diverse phenomena as "asynapsis", polytene chromosomes, somatic reduction, and diploid parthenogenesis; these will be discussed elsewhere. Here it may be pointed out that the absence of quadrivalents from isolated tetraploid nuclei, e.g. in *Podisma mikado* (38) and in *Forficula auricularia* (10) is not critical evidence that fusion of two diploid nuclei had occurred *after* pachytene (cf. 12, p. 65), but would be the natural consequence of syndiploidy following the establishment of two polarization centres at the anaphase of the last spermatogonial division.

** In the hermaphroditic coccid, Icerya purchasi, Dr. S. Hughes-Schrader (Z. Zellforsch. mikroskop. Anat. 6 : 509-540. 1927.) has shown that certain cells of the gonad undergo reduction and become haploid during the early divisions of the spermatogonia and that at spermatogenesis only one meiotic division occurs. No evidence on the cytological mechanism underlying this reduction was found, but it is clearly compatible with the scheme outlined here.*

Acknowledgments

It is a pleasure for the writer to state his gratitude to Prof. Franz Schrader and Prof. C. L. Huskins for expressions of opinion regarding the manuscript and to Dr. G. F. Sander for assistance with the literature.

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THE VITAMIN C (ASCORBIC ACID) CONTENT OF CANNED CHICKEN[1]

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Abstract

Vitamin C analyses on canned chicken indicate an appreciable amount in the product manufactured by improved methods. This amount, while low in proportion to that found in other foods, is sufficient to indicate the conservation of the vitamin by processes that minimize exposure to oxidation. The possible application of vitamin C analyses as an objective correlate to quality conservation in canning is discussed.

Introduction

Vitamin C is available in fresh and prepared fruits and vegetables, and also as synthetic *l*-ascorbic acid. Many additional foods contribute to the total received in average consumer diets. From a nutritional aspect a study of this factor in all foods, both raw and processed, is thus of value. Vitamin C is most unstable to oxidation, and to oxidation catalysed by heat or the presence of metals such as copper. Measurement of its retention during canning may indicate the degree to which oxidative changes in general have taken place. Thus, analysis for vitamin C may serve an additional purpose, as an index to quality preservation.

In conjunction with canning research on precooked poultry a number of analyses for ascorbic acid were conducted on both raw and processed products. Vitamin C studies reported herein are confined to canned precooked chicken, as the major marketed type of canned poultry. This commodity consists entirely of white and dark meat, and of broth prepared by cooking the whole cleaned and drawn birds, with head, feet, and all entrails removed.

Method

The method of analysis used throughout was the chemical titration procedure using 2-, 6-dichlorophenol indophenol as an oxidation–reduction indicator. The material was extracted with three separate portions of metaphosphoric acid using potassium cyanide as a stabilizer, and aliquots of the prepared extracts were titrated into the indophenol indicator, which was in turn standardized against pure synthetic *l*-ascorbic acid.

A number of concentrations of indicator were prepared in phosphate buffer at pH 7.2, and trial titrations on each extract enabled the choice of a concentration such that 1 ml. of indicator was decolorized to a decisive end-

[1] *Manuscript received May 1, 1942.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Published as Paper No. 77 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 1057.

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point by 5 ml. or less of the extract under assay. A titration of an extract from a micro-burette into a receiving flask containing 1 ml. of the chosen indicator, diluted to a convenient volume with distilled water, took an average time of one minute. By titrating aliquots of extract in which the vitamin C had been destroyed by oxidation, it was ascertained that no reduction of the indicator by reducing substances other than the vitamin, occurred in the titration time used.

Reduced ascorbic acid was determined by direct titration; dehydroascorbic acid was converted to the reduced form by treatment of aliquots of the extracts with hydrogen sulphide. Trial aliquots were used to determine the time necessary for maximum conversion to the reduced form. All residual hydrogen sulphide was completely expelled with nitrogen gas prior to retitration for the estimation of the dehydro form of the vitamin. Combined ascorbic acid (2) was not present in the processed product assayed, since it would be hydrolysed from combination by the heat treatments used in canning.

Results

The results given in Table I show the various amounts of vitamin C in raw meat, in broth from precooking, and in the final can. The amount of vitamin C in raw lean meat was found to be too low to establish any significant difference between grades in raw dressed poultry. Dark muscle meat was, however, definitely higher than white muscle meat.

TABLE I

VITAMIN C IN RAW AND PROCESSED CHICKEN MEAT*

Type of product	Number of samples	Vitamin C, mg. per 100 gm.		
		Reduced ascorbic acid	Dehydro-ascorbic acid	Total
1. Raw lean, white muscle meat	8	0.6 – 0.9 **(0.7)**	0.1 – 0.3 **(0.2)**	0.9 – 1.1 **(1.0)**
2. Raw lean, dark muscle meat	8	1.0 – 2.5 **(1.9)**	0.5 – 0.9 **(0.7)**	1.9 – 3.0 **(2.5)**
3. Precooking broth, pressure method	8	9.8 – 12.5 **(11.1)**	1.5 – 2.5 **(1.8)**	11.4 – 14.8 **(12.7)**
4. Precooking broth, open method	8	1.5 – 3.6 **(2.1)**	1.5 – 1.6 **(1.5)** (2 only)	3.4 – 3.6 **(3.5)** (2 only)
5. Final whole can, open precooked	17	2.4 – 4.7 **(3.3)**	0.1 – 0.5 **(0.3)**	2.6 – 5.1 **(3.6)**
6. Final whole can, pressure precooked	13	3.0 – 7.0 **(5.1)**	0.1 – 0.6 **(0.3)**	3.6 – 7.1 **(5.4)**

* *Whole digits only are significant, but decimal places are given for purposes of comparison.*

In the preparation of canned poultry there are three heating treatments. The first of these is a precooking, to prepare the broth, reduce the time necessary in retorting, and to allow separation of meat and bones. The other two heat treatments involve some method of exhausting the residual air from the can, and the commercial sterilization or retorting of the sealed can. The last two treatments are fairly well standardized in commercial production. Precooking may result in loss of quality unless conservational methods are used, and this process seems most amenable to improvement in the canning of poultry.

The method of precooking most widely used commercially is that of heating the poultry in open steam-jacketed kettles. Since the maximum temperature that can be obtained is boiling point, the time required to precook is lengthy, and there is continued loss of flavour and oxidation. Pressure precooking, using closed steam-jacketed kettles, was introduced as a more efficient method for the commercial production of canned poultry. A small volume of water within the kettle generates steam from heat applied by the outer jacket, and steam pressures giving temperatures of 250° F. or even higher may be used for the product within the kettle. Pressure kettles enable a rapid precooking with maximum conservation of flavour and food value.

Table I illustrates the differences, per unit volume, in the vitamin C content of poultry broth from open and pressure precooking. While open-kettle broth is considerably more dilute than that produced by the pressure method, the observed differences cannot be accounted for on this basis, and must be due to actual loss by oxidation in the former method. In this study the same aluminum inset was used for cooking by each method, while the times and temperatures were those developed to obtain maximum quality in commercial practice. In many instances the vitamin C content of precooking broth is higher per unit than the raw meat itself. The heating treatment in steam or boiling water is an efficient extraction process, transferring to the broth water-soluble constituents from the whole drawn birds, including portions that are discarded as scrap in the packaging of the canned commodity.

Table II contains the results of analyses on finished cans of sliced chicken. These data, as well as those given for canned poultry in Table I, were obtained from products processed by exactly the same procedure with the exception of the method of precooking. Analyses are given for canned chicken produced from the different grades of raw dressed chicken, and for some commercial packs. In all cases the product had been stored for 10 months after processing.

It will be noted that there is a trend towards a higher vitamin C content in the canned product from birds of higher dressed quality. The canned chicken produced by pressure precooking is somewhat higher in vitamin C content than that produced by open precooking, using the same grade of raw meat. Extensive quality test panels have shown a superiority in the canned product from higher grades of raw material. These test panels, conducted on a consumer basis, definitely showed that pressure precooked product is superior to

TABLE II

VITAMIN C ANALYSIS OF CANNED POULTRY*

Sample	No. of cans	Reduced ascorbic acid, mg./100 gm.	Dehydro-ascorbic acid, mg./100 gm.	Total ascorbic acid, mg./100 gm.	Total ascorbic acid, mg./7 oz. can
A. Open precook					
1. Grade special, milk-fed	3	4.1–4.8 (4.3)	0.0–0.3 (0.1)	4.2–4.8 (4.4)	8.3–9.5 (8.9)
2. Grade A, milk-fed	2	4.3–5.2 (4.7)	0.1–0.8 (0.4)	4.4–6.0 (5.2)	8.7–11.9 (10.3)
3. Grade B, milk-fed	3	3.0–3.5 (3.3)	0.0–0.6 (0.2)	3.4–3.6 (3.5)	6.7–7.1 (6.9)
4. Grade B, Lot I	3	2.5–3.0 (2.6)	0.0–0.6 (0.2)	2.6–3.1 (3.2)	5.1–6.1 (5.7)
5. Grade B, Lot II	3	2.2–2.9 (2.5)	0.0–0.2 (0.1)	2.2–3.1 (2.6)	4.4–6.1 (4.8)
6. Grade C	3	2.1–2.9 (2.4)	0.2–1.0 (0.5)	2.6–3.2 (2.9)	5.1–6.3 (5.8)
B. Pressure precook					
7. Grade special, milk-fed	3	5.7–6.2 (5.9)	0.0–0.3 (0.2)	5.8–6.3 (6.1)	11.5–12.5 (12.1)
8. Grade A, milk-fed	3	5.0–7.0 (5.6)	0.1–0.2 (0.16)	5.2–7.1 (5.8)	10.3–14.1 (11.5)
9. Grade B, milk-fed	3	4.5–5.1 (4.8)	0.2–0.7 (0.4)	4.9–5.8 (5.2)	9.7–11.5 (10.4)
10. Grade B	3	4.5–5.6 (4.8)	0.2–0.7 (0.4)	4.7–6.1 (5.3)	9.3–12.1 (10.5)
11. Grade C	3	2.5–3.3 (3.0)	0.5–0.6 (0.56)	3.1–3.9 (3.6)	6.1–7.7 (7.1)
C. Commercial packs					
12. Special pressure precook	2	5.2–5.2 (5.2)	0.0–0.6 (0.3)	5.2–5.8 (5.5)	10.3–11.5 (10.9)
13. Special open precook	3	3.0–3.7 (3.3)	0.0	3.0–3.7 (3.3)	5.9–7.3 (6.6)
14. Commercial I	3	1.3–3.1 (2.0)	0.0–0.4 (0.2)	1.4–3.1 (2.2)	2.8–6.1 (4.3)
15. Commercial II	3	1.5–1.9 (1.7)	0.0–0.2 (0.06)	1.5–2.1 (1.8)	3.0–4.2 (3.6)
16. Commercial III	3	1.8–2.7 (2.2)	0.0–0.1 (0.03)	1.8–2.8 (2.3)	3.6–5.5 (4.5)
17. Commercial IV	3	1.3–1.4 (1.36)	0.0–0.3 (0.16)	1.4–1.6 (1.5)	2.8–3.2 (3.0)
18. Commercial V	3	1.5–2.0 (1.8)	0.0–0.4 (0.2)	1.7–2.3 (2.0)	3.4–4.0 (3.6)
19. Commercial VI	3	1.9–2.4 (2.1)	0.0–0.2 (0.06)	1.9–2.4 (2.2)	3.8–4.8 (4.4)

** Statistical analysis of variance has shown a significant difference (necessary difference 0.9 mg.) between averages of grades as canned, and between canned product produced by pressure and open precooking. Both the latter are significantly higher than commercial canned precooked poultry.*

open precooked product, as judged by consumer preference. This preference was still apparent in packs showing no appreciable difference on chemical analysis for protein, fat, carbohydrates, and minerals.

Conclusions

The results of analyses show a higher content of vitamin C in canned poultry than in raw lean muscle meat, although only muscle meat is used in packaging. A considerable contribution to the total content comes from the broth prepared in precooking the whole drawn birds. The lower vitamin C content of sample cans from some commercial packs would indicate the need for more conservational processing.

Some correlation exists between quality and vitamin C conservation in canned chicken. The quality difference is not accountable for by ordinary chemical analysis, and the results offer a possible objective test for final quality based on vitamin C conservation. However, since the original vitamin C present in poultry meat is low, further detailed studies are planned on more suitable material in order that significant differences may be established throughout the entire canning processes used. Observations on vitamin C in the canning of fruit juices as made by Joslyn (1), indicate a close association of ascorbic acid and colour in citrus juices. Vitamin C analyses would appear to have practical application if a correlation between vitamin content and quality can be established in the food product concerned.

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A MATHEMATICAL ANALYSIS OF THE DISTRIBUTION IN MAIZE OF *HELIOTHIS ARMIGERA* Hb. (*OBSOLETA* F.)[1]

By Marjory G. Walker[2]

Abstract

The distribution among maize plants of the eggs of the American boll-worm, *Heliothis armigera* Hb., is discussed and analysed.

The problem is considered in relation to what is known of the connection between the state of development of maize plants and their attractiveness to ovipositing boll-worm moths. The actual frequency distribution of the eggs suggests a random as opposed to a uniform distribution, but it is shown that the conditions required for a pure mathematical random distribution cannot be satisfied. Because the maize plants differ from one another in absolute degree of attractiveness at any one time, and in relative degree of attractiveness with the passing of time, it is not true that every plant has the same chance of receiving any given egg.

It is demonstrated that a mathematical theory, which is eventually one of random distribution, but which incorporates a modification to allow for the varying degrees of attractiveness of the plants, gives a fairly good representation of the egg distribution found in the field.

Theoretical distributions to fit the data are calculated by two methods. One is a discontinuous process which is presented as only a rough approximation of what it is intended to express. The other uses the compound Poisson series of Greenwood and Yule. The continuous variation in nature both in space and time, which is the essential difficulty of the problem, is discussed.

Introduction

The problem of the distribution of animal populations is one that has been the subject of considerable discussion in recent years. The purely mathematical treatment of the question has outstripped the experimental verification of its theories and has, in consequence, forfeited the interest of the majority of biologists. Also, a plausible theory may be difficult or even impossible to verify, and until backed up by a suitable array of evidence it is at the mercy of any sceptic. The investigator who starts with facts is on surer ground, for then his hypotheses describe a series of possible events that *might* have produced the result actually observed. If the critic is now unable to improve matters by supplying a better idea, he must at least disprove the original assumptions, either by showing that they are not valid for an independent set of data, or by bringing forward evidence that has not previously been taken into consideration. In the present instance one of these possible objections has already been overcome because two separate sets of observations had been made on the same host-plant material. It was necessary, therefore, that the hypothesis developed in relation to one set should also be true for the other. This was found to be the case. Further, it was possible to interpret the

[1] *Manuscript originally received January 9, 1942, and in final form, May 14, 1942; submitted by Dr. W. R. Thompson, Imperial Parasite Service, Imperial Agricultural Bureaux, now stationed at Belleville, Ont.*

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mathematical relation that was found to connect the two analyses in terms of what is actually known to happen in the field. There is thus some justification for this particular method of dealing with population problems.

The Problem and the Data

Marshall (2) published an account of the distribution and sampling of populations of the American boll-worm, *Heliothis armigera* Hb. (*obsoleta* F.). Because there is a high mortality among the larvae, particularly in the first and second instars, and because of their migratory movements, the most convenient indicator of the progress of an infestation has been found to be the oviposition of the moths. Parsons and Ullyett (3) had already shown that eggs laid by boll-worm moths are most unevenly distributed throughout a crop. Marshall's object was therefore primarily to determine the nature of the egg distribution and secondly to devise a method of sampling a crop so as to avoid inaccuracies due to the variation in the number of eggs. The present paper is a development of the first part of the problem[1].

Among the wide variety of crops attacked, maize is particularly favoured by the American boll-worm, oviposition beginning approximately with the appearance of the first tassels and reaching its peak by the time tassellation has occurred on all plants. Accordingly, for his experiment Marshall chose a field of drill-planted maize which was just coming into the attractive stage. A plot (23 × 24 sq. yd.) was taken at random in this field. As the maize was planted in rows a yard apart, there were in the plot 23 rows each 24 yd. long. The exact position of each of the 782 maize plants was measured and recorded in the form of a scale diagram. In Marshall's own words: "The experiment consisted of counting the eggs on every plant on two occasions with an interval of one week between the counts. The eggs of the moth are very minute, but the work was carried out by specially trained natives under white supervision with constant checking. The nature of the work makes a small error unavoidable, but this would certainly be no more than usually occurs in the course of the ordinary routine sampling. An individual count was conducted within the space of one day, and the eggs counted were crushed so that there could be no possibility of the second count being in error through the addition of old eggs." The two counts were recorded plant by plant. A summary of the result is given in Table I.

[1] *Note by Dr. W. R. Thompson.*

Dr. J. W. Hopkins of the Division of Applied Biology, National Research Laboratories, Ottawa, Canada, has very kindly pointed out that in 1938 a paper entitled "Discrete frequency distributions arising from several single probability values", was published in the Journal of the American Statistical Association, 33 : 390-398, by Dr. H. Muench. In this paper, Dr. Muench gave a solution, by the method of moments, of one of the hypotheses considered by Dr. Walker in connection with the oviposition of Heliothis, i.e., that there is a relatively small number of distinct categories of attractiveness, within each of which oviposition occurs at random, with a probability that is constant within each specific category but differs from one category to another. However, Dr. Walker's work had been completed and her paper prepared for publication before Dr. Muench's article appeared in print. She is now engaged in war work in Scotland and further revision of the paper at this time is impracticable.

TABLE I

No. of eggs per plant	No. of plants		No. of eggs per plant	No. of plants	
	1st Count	2nd Count		1st Count	2nd Count
0	206	101	17	2	11
1	143	72	18	1	7
2	128	89	19	–	1
3	107	84	20	–	3
4	71	66	21	–	1
5	36	40	22	–	2
6	32	56	23	–	2
7	17	43	24	–	1
8	14	49	25	–	–
9	7	28	26	–	1
10	7	39	27	–	1
11	2	13	28	–	–
12	3	17	29	–	2
13	3	12	30	–	–
14	1	14	32	–	1
15	1	12	34	–	3
16	1	10	51	–	1

Marshall says "The distribution for both counts was very similar. The biological aspect of the inquiry, as well as the nature of the distribution curves, suggested a Poisson series, but all attempts to fit such were unsuccessful. This might have been accounted for by the drawn-out tail of the curves where a small number of plants with high egg counts were recorded. Also the minuteness of the eggs and their often hidden position on the plant introduced a considerable error into the zero and few egg per plant classes. Such error would preclude any satisfactory fit." It is rather difficult to accept this explanation of a possible source of error in the egg counts. When an individual is set a task such as this one of counting eggs some of which are difficult to find, if he comes across a plant on which they are numerous he may quite easily lose count and record the number as too few or too many. On the other hand, if he is examining a plant but is unable to find any eggs on it, the fact that his job is to count eggs is a stimulus for him to keep on searching and to make sure of not missing any. From the psychological point of view, then, it is unlikely that errors of counting would be concentrated among the classes of plants with a few or no eggs, and in the present analysis it will be assumed that the counts were made with reasonable accuracy. On the other hand, the figures of the second count suggest the possibility of a bias in favour of even numbers. In Fig. 2 the graph of the data shows peaks at 6, 8, and 10 eggs per plant. Yule (4) discusses the idiosyncrasy in the case of reading scales. He points out that where a fraction of a unit has to be judged by eye, there is a tendency in some observers to round it off to 0.0 or 0.5 while in addition there is a preference for fractions expressed by an even digit as opposed to those expressed by an odd digit, with the exception of 0.5. Reading a scale is an entirely different proposition from that of searching for and counting objects. However a comparable feature in the collection of the

present data might be that a number of the searchers tended to be more satisfied that their efforts had been thorough and sufficient when they had found an even number of eggs on a plant than when they had found an odd number. In any case the biological evidence is that the eggs are laid singly and not in pairs. If there has been a particular bias in the recording of the numbers of the eggs in this investigation, it is begging the question to suppose that we can make any assessment of its effect. The recorded numbers of eggs are the results of a combination of the biological factors governing the distribution of the eggs, which is what we want to find out, and the human factors influencing the counting of the eggs. There are not sufficient data to allow the two to be dissociated. It can never be proved that the *recorded* distribution of the *Heliothis* eggs is other than what did in fact occur, and the present writer can only accept Marshall's statement that the observations were made with all due care and with constant checking. The assumption that the best fit for the peaks in question is a smooth curve running through them has been made independently of considerations of their possible cause.

As Marshall points out, the most likely biological hypothesis is that the boll-worm eggs would be distributed at random among the maize plants. The eggs are laid singly and are to be found in various parts of the plant. If it were known that the heavy mortality among the young larvae is due to overcrowding on heavily infested plants, then one would expect that the moths might show a tendency to oviposit to the advantage of their progeny, i.e., so that there would be signs of some attempt at a uniform distribution among the the plants. However, the records given above of the egg counts completely fail to suggest that any such faculty is put into use by the insects. If the boll-worm eggs are distributed among the maize plants at random in the strictly mathematical sense, then it must be true that the chance of any particular egg being laid on one specified plant is exactly the same as its chance of being laid on any *other* specified plant. If for any reason this condition is not quite satisfied, then obviously the frequency distribution of the eggs among the plants will not fit the calculated theoretical distribution. In the present paper a modification of the simple random distribution is suggested. This is based on the field observations of Parsons and Ullyett (3) and it is shown that with its aid theory can be made to fit facts.

The Method of Analysis

The object of the following illustration is to give the reader a clearer mental picture of random distribution, and particularly of the modifications that concern the present problem. Imagine an open oblong box. A person dropping marbles into it would probably tend to hold each marble somewhere over the middle of the box, but if he omitted this precaution, or better still, if he did not mind that some of the marbles missed the box altogether, then those marbles that fell into the box could be said to hit the bottom at random. Suppose that a hundred small boxes, in, say, 10 rows of 10, fitted exactly into the bottom of the large box, and that they were the same depth as their

container. If the small boxes were all of the same size, each box would have exactly the same chance, one in a hundred, of receiving any particular marble that fell within the big box. The marbles in the big box would then be distributed at random among the hundred little boxes. When the total number of marbles in the big box is known, it is easy to calculate the probable number of compartments that remain empty, or contain 1, 2, 3, etc. marbles.

Now suppose that the hundred small boxes, although all of the same height, are not all of the same size. They still fit into and exactly fill the bottom of the big box, but 25 of them are twice as large as the remaining 75. The marbles would still fall at random into the big box, and they would still *fall* at random into the hundred compartments of the box, but they would not be *received* at random by these compartments in the same sense as in the previous example. A *particular* large compartment is now twice as likely as a *particular* small compartment to receive any marble that falls into the big box. This complicates the calculation of the probable distribution of the marbles in the compartments. One method of overcoming the difficulty is to treat the distributions in the small and large compartments separately. It was postulated that the bottom of the large box was entirely covered by the 75 small compartments and the 25 larger compartments, each of the latter occupying double the basal area of the former. The area of the bottom of a small compartment being taken as a unit, there are 125 units in the area of the base of the large box of which 75, or three-fifths of the total are occupied by the small compartments, and 50, or two-fifths by the larger ones. It would therefore be expected that three-fifths of the marbles falling at random into the box would be received by the small compartments and two-fifths by the large compartments. When the total number of marbles in the box is known the two calculations required are the random distribution of three-fifths of that number among 75, and of two-fifths of the number among 25, from which the probable numbers of compartments with 0, 1, 2, 3, etc. marbles may be obtained as before. This, as will be explained later, corresponds in a simplified way to the first modification of random distribution required in the treatment of the present problem.

For the second modification imagine the large box as before with its hundred compartments all of the same size. After a certain fraction of the marbles have fallen at random into the box, suppose that 10 compartments, the end row for example, are removed and replaced by a fresh lot. Another fraction of the marbles falls into the box and this time the second row of compartments is renewed, a third fraction of marbles falls and then the third row is renewed, and so on. The marbles are all the time falling at random into the box, and they are in every case each being received by any one at random of a hundred similar compartments, though the individuals that comprise this total of a hundred change as time goes on. The calculations for the probable numbers of compartments with 0, 1, 2, 3, etc. marbles must be worked out each time 10 new compartments are substituted for 10 of the original ones. They are extremely laborious, but not more difficult mathematically than the essential random distribution calculation.

The hypothesis that is the basis of the explanation suggested for the distribution of *Heliothis* eggs among maize plants is that the changes that occur in the field as a crop matures could be likened to the theoretical cases just described.

Parsons and Ullyett (3) demonstrated, among other facts, the very great attractiveness of maize crops, at a certain rather limited phase of their development, to ovipositing *Heliothis* females. Here is what they observed at one farm: "There were four fields of maize which had been planted two or three weeks apart. The total acreages of maize and cotton were practically equal. Eggs of *H. obsoleta* were found first on the earliest crop of maize. At the time oviposition was first observed to occur on these maize plants, 10 per cent of them had just extruded the tip of the tassel or male inflorescence. The plant cotton at the time was flowering freely, and ratooned cotton bore fresh bolls and flowers in abundance. Cotton in this condition is considered to be highly attractive to *H. obsoleta*." Their data show, however, that "all the oviposition occurred in successive waves on the successive maize crops" (from mid-December till the beginning of March). "No *Heliothis* eggs were found on cotton on this farm until mid-March." Of another farm where there was one planting of maize and one main field of cotton they say:— "The maize crop was planted some weeks after cotton and experienced very difficult, low moisture conditions, in consequence of which growth was delayed and fruiting meagre. . . . . considerable oviposition was recorded on cotton before eggs were found on maize, but . . . . . when eggs appeared on the maize plants, egg-laying on cotton ceased and was not resumed until that on maize fell off. Thereafter renewed oviposition was recorded on cotton. In this instance it was observed again that *Heliothis* commenced laying on maize simultaneously with the appearance of the tassels. Although the maize plants were very poorly grown, they were not, apparently, unattractive to the bollworm moth. The maize and cotton lands were adjacent." On a third farm:— "The rows of maize were situated alongside the cotton. Records were taken in the strip of maize plants and the cotton concurrently. While oviposition was in progress on the maize, eggs were not found on the adjacent cotton. Immediately oviposition on the maize plants fell off, eggs were recorded on cotton . . . . . In this instance also an early and heavy oviposition had occurred on cotton prior to the commencement of recording and before the maize plants were in tassel as numbers of larvae were feeding on the cotton when observers first entered the crop."

"On three other farms similar conditions were observed. If maize was present as well as cotton, *Heliothis* at some period in the season practically confined oviposition to maize. From growth records it was observed that a definite relationship appeared to exist between egg deposition on maize and certain conditions in the reproductive phase of this plant."

As the quotations show, Parsons and Ullyett were very emphatic about "the coincidence between the appearance of eggs in numbers on maize and the production of the male inflorescence." This was because the general

implication of the earlier literature had been that the maize plants are most attractive to the moths during the production of the female flowers (silks) which appear after the extrusion of the tassels, or less precisely, that they are attractive from the time they are about 16 in. high until the leaves begin to turn yellow, a period of several months. To make their evidence more conclusive they made daily counts of eggs on a number of marked maize plants, which were examined twice weekly for height and for the development of leaves, tassels, and silks. The data, presented in an interesting diagram, show the striking correlation between oviposition and tassellation. "Only a very few eggs were found before the first tassels appeared. Thereafter eggs became abundant, and the peak of the oviposition coincided with tassellation on all plants. Up to that time 70 per cent of the total eggs laid had been recorded." "During the main period of abundant, fresh silk production, egg-laying did not occur."

"This coincidence of insect–plant behaviour is being investigated at the present time with regard to certain chemical changes in the plant which are associated with the reproductive phase."

As has already been pointed out, oviposition by *Heliothis* among the maize plants can be strictly random only if every plant has the same chance of receiving any particular egg, but, as the field evidence has shown over and over again, oviposition is associated with the appearance of the tassels. Now, in the diagram already mentioned, it can be seen that among the plants specially observed by Parsons and Ullyett, the first appearance of tassels continued in the different plants over a period of 13 days. It would thus seem to be a reasonable hypothesis that the plants are not all equally attractive at any one time. If this idea is accepted it must be at once obvious that the arrangements of eggs among the plants cannot be comparable to the elementary random distribution. This brings us to the application of the first of the modifications explained by the example of marbles dropping into compartments. When some compartments are larger than others, they are more likely to receive marbles. In the same way the maize plants that protrude their tassels earliest may, to begin with, be more attractive than the other plants. If Parsons and Ullyett are right when they suggest that the attractiveness of maize to *Heliothis* is due to chemical changes that take place in the plants at a certain phase in their development, then it is the early development of individual plants and not the crop as a whole that is responsible for the commencement of oviposition in a maize field and the corresponding cessation in a neighbouring cotton field.

Now although Parsons and Ullyett kept a record of the development of the individual plants on which they made the daily egg counts, they do not mention that they found more eggs on plants in some particular stage of tassel development than on those that were less mature at the time. But supposing that out of a hundred maize plants, 50 of them, being at a more attractive stage, were twice as attractive as the remaining 50. Then on an analogy with the 50 large and 50 small compartments, if a total of 600 eggs were laid, the

50 attractive plants should receive 400 eggs and the others only 200 eggs. But if the 400 eggs were distributed at random among the 50 plants—and there is no obvious reason why they should not be—then according to the mathematical calculation we should expect that some of these plants would receive quite small numbers of eggs, as well as others receiving large numbers. In the same way in the distribution at random of 200 eggs among 50 plants, a certain proportion of the plants would probably receive quite large numbers of eggs. This overlapping of the two distributions would tend to obscure the law that plants at a certain stage of maturity attain their maximum phase of attractiveness and receive *on the average* the greatest number of eggs from the ovipositing moths.

There were 782 maize plants in Marshall's experimental plot. The first count of the *Heliothis* eggs gave a total of 1934, an average of 2.47 eggs per plant. As Marshall pointed out, the egg frequency distribution of the field data differs from the theoretical distribution resulting from the calculation of the random distribution at the rate of 247 eggs per 100 plants, in having too many plants with no eggs and with very few eggs, and again too many plants with high numbers of eggs. After a number of trials it was found that on the assumption that $\frac{4}{7}$ of the plants received eggs at random at the average of 247 eggs per 100 plants (the average for the whole plot) that $\frac{2}{7}$ of the plants received eggs at random at the average of 30 eggs per hundred plants, while the remaining $\frac{1}{7}$ of the plants received at random an average of 681 eggs per 100 plants, the theoretical egg distribution in the plants taken as one group bears a satisfactory resemblance to the real distribution. The second, third, and fourth columns of Table II show the random distributions of 30, 247, 681 respectively in 100, and the fifth column shows the theoretical egg frequencies among the plants when 247 eggs are distributed per 100 plants in the manner outlined. This column is calculated from the other three columns line by line and it may be represented by the general formula:

$$\{\tfrac{2}{7} \times (R.D. \text{ of } 30 \text{ in } 100)\} + \{\tfrac{4}{7} \times (R.D. \text{ of } 247 \text{ in } 100)\} + \{\tfrac{1}{7} \times (R.D. \text{ of } 681 \text{ in } 100)\},$$

where *R.D.* stands for Random Distribution and is calculated from the terms of the Poisson distribution, namely

$$Ne^{-m}\left(1 + m + \frac{m^2}{\underline{|2}} + \frac{m^3}{\underline{|3}} + \ldots\ldots\right),$$

where $N = 100$, the number of plants and m, the hypothetical arithmetic mean of the numbers of eggs per plant in the various groups, has the values 0.30, 2.47, and 6.81. The theory underlying the use of this formula is given in most textbooks on statistics; e.g. Yule and Kendall (5). In Fig. 1, also, the actual and theoretical distributions of the eggs are shown, the scale being adjusted to represent the total 782 plants. It differs from Table II in that the three component curves that represent the egg distribution in the three grades of plants are shown in the same relative scale, and the sums of their ordinates give the ordinates of the curve for the whole population.

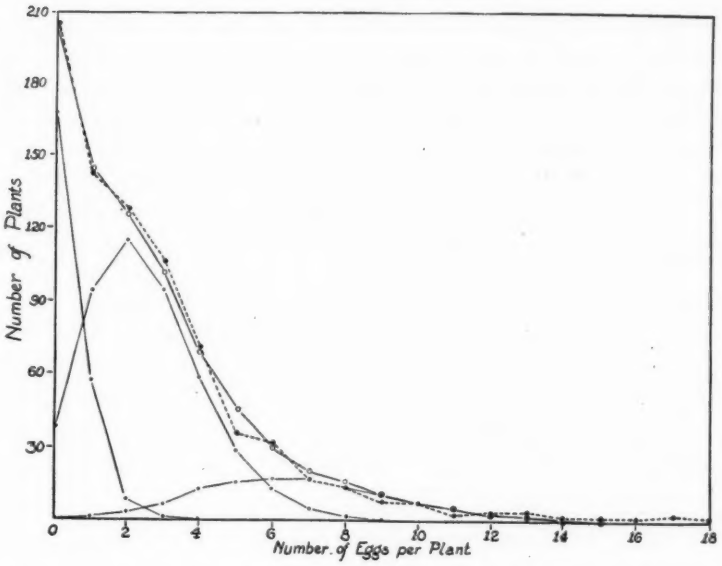

FIG. 1. *First egg count and theoretical distribution obtained by summing three random distribution series;* ●- - - -● = *data,* ○——○ = *theoretical distribution,* ●——● = *component curves.*

TABLE II

No. of eggs per plant	Random distributions in 100			Theoretical distribution	1st Count per 100 plants
	30	247	681		
0	74.082	8.458	0.110	26.02	26.34
1	22.225	20.892	0.751	18.40	18.29
2	3.337	25.802	2.557	16.06	16.37
3	0.334	21.243	5.804	13.06	13.68
4	0.025	13.118	9.882	8.92	9.08
5		6.480	13.459	5.63	4.60
6		2.668	15.276	3.71	4.09
7		0.941	14.861	2.66	2.17
8		0.291	12.651	1.97	1.79
9		0.080	9.572	1.41	0.90
10		0.020	6.519	0.94	0.90
11		0.004	4.036	0.58	0.26
12			2.290	0.33	0.38
13			1.200	0.17	0.38
14			0.584	0.08	0.13
15			0.265	0.04	0.13
16			0.113	0.02	0.13
17			0.045	0.01	0.26
18			0.017		0.13
19			0.006		
20			0.001		

The second count was made a week after the first, and, as has been mentioned the numbers of eggs recorded were quite independent of those in the first count. On this occasion 4499 eggs were present on the 782 plants making an average of 5.75 eggs per plant. The curve formed by the egg distribution (Fig. 2) is rather irregular, but a fairly good fit was obtained from the assumption that $\frac{5}{14}$ of the plants received eggs at random at the average of 746 eggs per 100 plants, $\frac{5}{14}$ received eggs at random at the average of 247 eggs per 100 plants, $\frac{1}{7}$ received eggs at random at the averages of each of 50 and 1492 eggs per 100 plants. Table III shows each of these distributions and also the distribution for the whole population calculated from the formula:

$$\{\tfrac{1}{7} \times (R.D. \text{ of } 50 \text{ in } 100)\} + \{\tfrac{5}{14} \times (R.D. \text{ of } 247 \text{ in } 100)\} + \{\tfrac{5}{14} \times (R.D. \text{ of } 746 \text{ in } 100)\} + \{\tfrac{1}{7} \times (R.D. \text{ of } 1492 \text{ in } 100)\}.$$

The four component curves are shown in scale in Fig. 2. No theoretical curve based on hypotheses of random distribution can be constructed to follow the sharp angles of the data curve. The one given does, however, represent quite well a smoothed curve of the data.

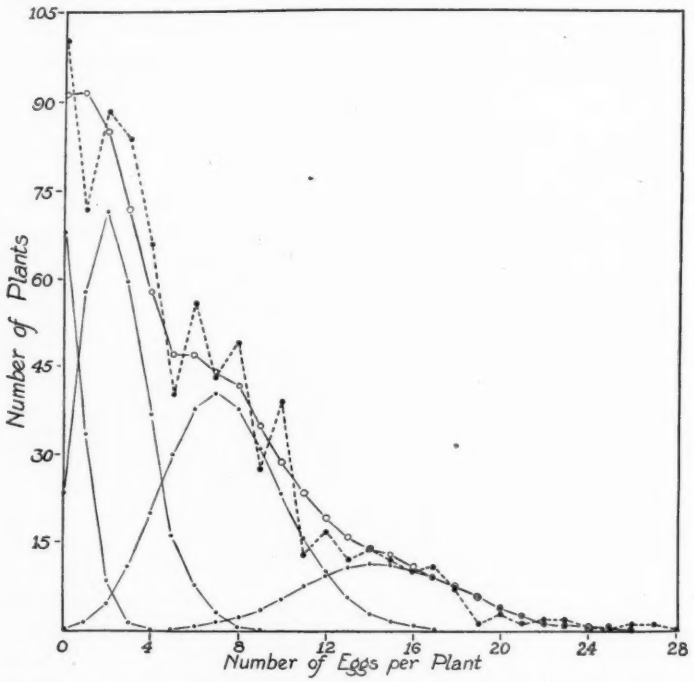

FIG. 2. *Second egg count and theoretical distribution obtained by summing four random distribution series;* ●----● *= data,* ○——○ *= theoretical distribution,* ●——● *= component curves.*

TABLE III

No. of eggs per plant	Random distributions in 100				Theoretical distribution	2nd Count per 100 plants
	50	247	746	1492		
0	60.665	8.458	0.058	0.000	11.71	12.92
1	30.326	20.892	0.429	0.000	11.95	9.21
2	7.582	25.802	1.602	0.004	10.92	11.38
3	1.264	21.243	3.983	0.018	9.19	10.74
4	0.158	13.118	7.429	0.068	7.37	8.44
5	0.016	6.480	11.083	0.204	6.30	5.11
6	0.001	2.668	13.780	0.507	5.96	7.16
7		0.941	14.686	1.082	5.74	5.50
8		0.291	13.695	2.018	5.28	6.27
9		0.080	11.351	3.345	4.56	3.58
10		0.020	8.468	4.991	3.74	4.99
11		0.004	5.743	6.771	3.02	1.66
12			3.570	8.418	2.48	2.17
13			2.049	9.661	2.11	1.53
14			1.092	10.296	1.86	1.79
15			0.543	10.242	1.66	1.53
16			0.253	9.550	1.45	1.28
17			0.111	8.381	1.24	1.41
18			0.046	6.948	1.01	0.89
19			0.018	5.456	0.79	0.13
20			0.007	4.070	0.58	0.38
21			0.002	2.892	0.41	0.13
22			0.001	1.961	0.28	0.26
23				1.272	0.18	0.26
24				0.791	0.11	0.13
25				0.472	0.07	—
26				0.271	0.04	0.13
27				0.150	0.02	0.13
28				0.080	0.01	—
29				0.041		0.26
30				0.020		—
31				0.010		0.26
32				0.005		—
33				0.002		—
34				0.001		0.38
51						0.13

Having given a possible explanation of the egg distribution found in the first and second examinations of the maize plants we should now be in a position to discuss the sum of the two distributions in terms of the same theory. Marshall dealt with the resemblances and differences between the two egg counts but not with their sum. However, since his records preserved the individuality of each plant it was a simple matter to add together the numbers of eggs found on the two occasions and so to obtain the frequency distribution for the grand total of eggs observed (Table IV, final column). This could have been dealt with in the same manner as were the two previous distributions, by dividing the number of plants into groups representing different degrees of attractiveness, and working out a new series of random distributions to correspond. But since the real distribution of the sum was obtained from two real distributions it is more satisfactory that the theoretical distribution for the sum should also be derived from the two theoretical distri-

TABLE IV

No. of eggs per plant	Random distributions									Theoretical distribution	Sum of two counts
	$\frac{3}{2\,8}$ at 80 per 100	$\frac{2}{2\,8}$ at 277 per 100	$\frac{8}{2\,8}$ at 494 per 100	$\frac{1}{2\,8}$ at 731 per 100	$\frac{3}{2\,8}$ at 776 per 100	$\frac{5}{2\,8}$ at 993 per 100	$\frac{2}{2\,8}$ at 1427 per 100	$\frac{3}{2\,8}$ at 1739 per 100	$\frac{1}{2\,8}$ at 2173 per 100		
0	37.65	3.50	1.60	0.02	0.04	0.01				42.82	45
1	30.12	9.69	7.90	0.14	0.28	0.07				48.20	54
2	12.05	13.43	19.50	0.50	1.08	0.34				46.90	50
3	3.21	12.40	32.12	1.22	2.78	1.11	0.02			52.86	54
4	0.64	8.59	39.66	2.22	5.40	2.75	0.06	0.01		59.33	61
5	0.10	4.76	39.19	3.25	8.38	5.47	0.17	0.03		61.35	68
6	0.01	2.20	32.27	3.96	10.84	9.05	0.42	0.09		58.84	53
7		0.87	22.77	4.13	12.02	12.84	0.85	0.22		53.70	50
8		0.30	14.06	3.78	11.66	15.94	1.51	0.49	0.01	47.75	39
9		0.09	7.72	3.06	10.05	17.59	2.40	0.94	0.03	41.88	46
10		0.03	3.81	2.24	7.80	17.47	3.42	1.64	0.07	36.48	31
11		0.01	1.71	1.49	5.50	15.77	4.44	2.59	0.13	31.64	26
12			0.70	0.91	3.56	13.05	5.28	3.75	0.24	27.49	26
13			0.27	0.51	2.12	9.97	5.79	5.02	0.39	24.07	36
14			0.10	0.27	1.18	7.07	5.91	6.24	0.61	21.38	27
15			0.03	0.14	0.60	4.68	5.62	7.23	0.89	19.19	15
16			0.01	0.07	0.29	2.90	5.01	7.85	1.20	17.33	16
17				0.03	0.13	1.70	4.21	8.03	1.54	15.64	8
18				0.01	0.06	0.94	3.33	7.76	1.86	13.96	13
19					0.02	0.49	2.50	7.11	2.13	12.25	15
20					0.01	0.25	1.79	6.18	2.31	10.54	9
21						0.12	1.21	5.12	2.39	8.84	7
22						0.05	0.79	4.05	2.36	7.23	4
23						0.02	0.49	3.05	2.23	5.79	5
24						0.01	0.29	2.21	2.02	4.53	3
25							0.17	1.54	1.76	3.47	4
26							0.09	1.03	1.47	2.59	8
27							0.05	0.66	1.18	1.89	—
28							0.02	0.41	0.92	1.35	—
29							0.01	0.25	0.69	0.95	1
30								0.14	0.50	0.64	—
31								0.08	0.34	0.42	1
32								0.04	0.24	0.28	1
33								0.02	0.16	0.18	1
34								0.01	0.10	0.11	1
35									0.06	0.06	—
36									0.04	0.04	1
37									0.02	0.02	—
38									0.01	0.01	—
39											—
40											—
42											1
48											1
52											1

butions. The fact of this being possible is an additional justification for the hypotheses used.

Marshall says: "*A priori* it was to be expected that over a short period of time, as between the two counts taken in this experiment, that the crop should retain its inherent differences in growth and attractiveness." One would agree that if, on a certain date, some plants were more advanced and some

less advanced than others, then it is to be expected that a few days later they would still be in the same *relative states of development;* but an opinion as to their expected *relative states of attractiveness* on the two occasions would need to be based on a more precise knowledge of what causes maize plants to become attractive to *Heliothis*. Referring to the arrangement of the plants in rows and to a method of assessing the egg distributions by taking the total number of eggs for each individual yard length of the rows, Marshall continues: "A correlation trial was worked out between the first count per yard and the second count on the same yard. (All yard lengths having no plants in them were removed from the analysis.) A significant correlation coefficient was found of +0.3348 with 454 degrees of freedom. This was highly significant at the 0.01 level, and the validity of the correlation was established. A similar correlation on egg counts *per plant* on the two occasions also showed a positive correlation between the two counts. It was therefore definitely established that the same portions of the crop tended to retain their relative attraction to the boll-worm moth over short periods of time".

In the present paper the main assumption underlying the analysis is that the plants are not all equally attractive to the moths. The basis for this assumption is the evidence of Parsons and Ullyett that oviposition starts when the first plants begin to show the tassels, and continues at a rapidly *increasing rate* as more and more plants show their tassels. The maximum number of eggs is present at the time when all the plants have protruded tassels, but the *rate* of increase in the number of eggs begins to *diminish* some days earlier at the period when the proportion of plants with fully extruded tassels is showing a rapid increase. The natural inference is that the first plants to have their tassels full out are those that originally attracted the first moths to the field. The first plants to be most attractive would be the first to pass the period of greatest attractiveness, and hence would be responsible for the falling off in the rate of increase in the number of eggs. The tendency for a positive correlation between egg counts on individual plants at an interval of as much as a week would therefore seem to depend on the relation of the time when the first count was made to the whole period of oviposition. If the first count were made early in the oviposition period, the second count, a week later, would be made while all the plants were still, on the whole, increasing in attractiveness. On the other hand, if the first count were made just before the peak of oviposition, when none, or only a few of the plants had passed out of the most attractive stage, then a week later a much higher proportion of plants would have passed from the most attractive to a less attractive stage. In a case such as the latter the correlation between the two egg counts, if still positive, would certainly be less significant than in a case of the former type.

The theoretical distribution for the sum of the two counts was calculated first of all on the hypothesis that the plants that were very attractive in the first count were still the most attractive in the second count. The basic random distributions that had to be worked out were therefore as follows:

Fraction of population	No. of eggs received per 100 plants		
	1st Count	2nd Count	Total
$\frac{2}{14}$	30	50	80
$\frac{2}{14}$	30	247	277
$\frac{3}{14}$	247	247	494
$\frac{5}{14}$	247	746	993
$\frac{2}{14}$	681	1492	2173

Fig. 3 shows these five random distributions and the curve which is the sum of their ordinates. In comparison with the real distribution it is seen to have too high proportions of plants in the groups with very many and very few eggs. After several trial rearrangements the best fit was obtained from the one given below:

Fraction of population	No. of eggs received per 100 plants		
	1st Count	2nd Count	Total
$\frac{3}{28}$	30	50	80
$\frac{2}{28}$	30	247	277
$\frac{3}{28}$	30	746	776
$\frac{6}{28}$	247	247	494
$\frac{4}{28}$	247	746	993
$\frac{3}{28}$	247	1492	1739
$\frac{1}{28}$	681	1492	2173
$\frac{2}{28}$	681	746	1427
$\frac{1}{28}$	681	50	731

The details of these nine random distributions are given in Table IV where, for convenience, they have been worked out to the requisite fractions of 782, the total plant population of the sample plot. The theoretical curve in Fig. 4, while not a particularly good fit, is better than that in Fig. 3, and, considering the irregularities of the real curve and the limitations of the method of calculation, it is probably as much as could be expected from the nature of the hypotheses.

The most important features of the curve in Fig. 4 are, firstly, that the group of plants that is most attractive in the second egg count includes only one-fourth of those that were most attractive in the first count, the remainder being derived from those that were only moderately attractive; secondly, the remainder of the highly attractive group in the first count is considered to have passed the peak of attractiveness so that its total of eggs recorded in the second count is considerably less than the maximum. In particular one-fourth of the group is supposed to have passed almost entirely out of the attractive phase, while the remaining half of the group is still quite strongly attractive. "Group" is here used in connection with the mathematical and not the spatial aspect of the problem. This is the second modi-

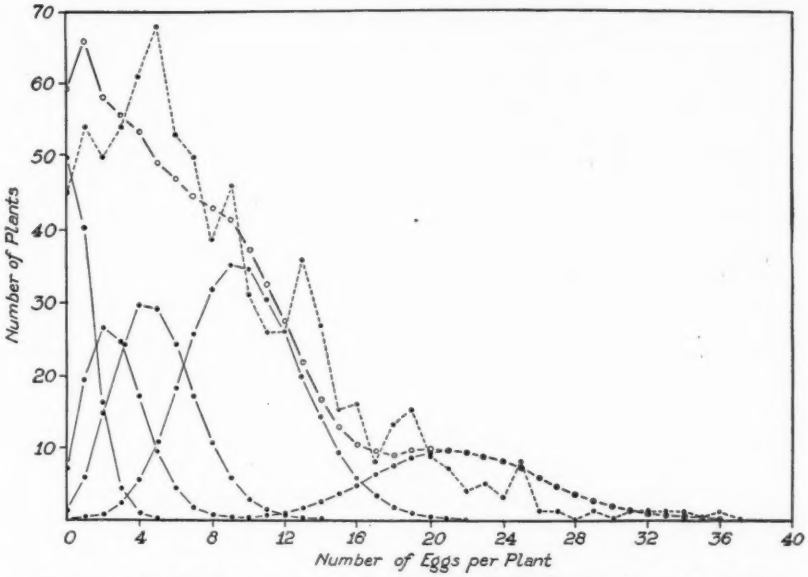

FIG. 3. *Sum of the two egg counts and a theoretical distribution based on the assumption that the plants have retained, more or less, the same relative attractiveness to the moths at both periods;* ●- - - -● *= data,* ○———○ *= theoretical distribution,* ●———● *= component curves.*

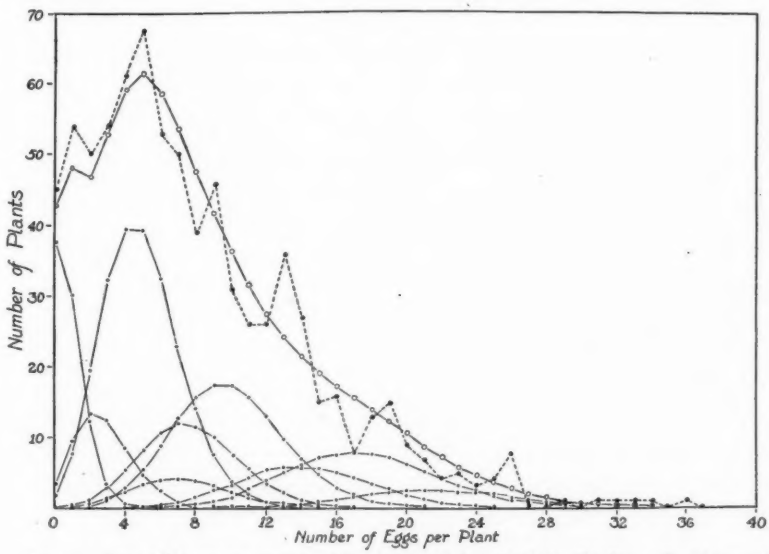

FIG. 4. *Sum of the two egg counts and an improved theoretical distribution. In this case it is assumed that the plants, by their development in the interval between the two counts, have altered in their relative attractiveness to the moths;* ●- - - -● *= data,* ○———○ *= theoretical distribution,* ●———● *= component curves.*

fication of sample random distribution which was illustrated by the theoretical case of dropping balls into boxes. A set of boxes represents a particular group of plants, the most attractive plants, for example, or the least attractive. As the balls drop some boxes are added to the group, others are removed. So with the plants, as time passes, new plants reach the most attractive phase while others continue their development beyond that phase and become classified along with less attractive plants in another group.

The various groups or types of plants are considered to be mixed indiscriminately throughout the plot. In the first egg count, a large fraction of the plants, $\frac{2}{7}$, was considered to be attractive in only a very slight degree. During the course of the week it would be expected that these plants would have developed and become more to the liking of the moths. The interpretation, according to the accepted calculation for the sum of the counts, is that three-eighths of that group ($\frac{3}{2\,8}$ of the population of the plot) was still almost entirely neglected by the moths, in spite of the greatly increased rate of oviposition. This could be due to the relative backwardness of the plants, or because some or all of them were in an unhealthy condition from some other causes. The remainder of this group is assumed to have developed normally according to expectation, such that one-quarter became moderately attractive and the other three-eighths more so. A similar fate in the second count is shown for the large group of plants that were moderately attractive in the first count. To avoid confusion it should be remembered that the progress of tassellation in the field as a whole may safely be assumed to have attracted more and more moths to that field. There was thus a much higher rate of egg-laying during the week between the counts than during the week previous to the first count, and therefore the numbers of eggs allocated to the group of plants designated as moderately attractive, most attractive, etc., were proportionately very much higher in the second count than in the first.

The Limitations of the Method

In the preceding section, field data on the distribution of *Heliothis* eggs among maize plants have been analysed with the aim of explaining how that particular numerical arrangement could have come about. The explanation was based on recorded observations on the relation between the development of maize plants and their attractiveness to ovipositing *Heliothis* moths. The original and most simple theory was that the female moths distributed their eggs at random among the plants, and Marshall tested the idea by comparing his data with a random distribution calculated from a Poisson series. The reason why this theory failed to agree with the facts was quite plain from the work of Parsons and Ullyett. These workers showed that not only was it true in the general sense, that a maize crop becomes attractive to the moths when the tassels are beginning to appear, but that more particularly the increasing degree of oviposition can be correlated with the rising percentage of tassels that appear, and further that the percentages of tassels fully developed and of silks showing are associated with the subsequent dwindling of

the oviposition rate in the crop. This rising and falling in the intensity of oviposition does not correspond to the normal egg-laying cycle found in the cases of insects with isolated generations all the individuals of which reach the same stage of development at approximately the same time. The *Heliothis* moths oviposit in other crops before and after a maize crop is in the desired stage of maturity, and so the peak of egg-laying activity in a particular field of maize represents the time when the greatest numbers of moths are present. If it had been obvious to Parsons and Ullyett from their daily examinations of the individual plants that the moths showed a special preference for a particular stage of development, they would undoubtedly have stressed the point in more detail than by merely discussing the trend of oviposition in its relation to the crop as a whole. Since the distribution of eggs among the plants certainly does not suggest uniformity, one therefore naturally returns to the idea of a random distribution, adjusted to include the concept of the non-uniformity of the plants. It has already been pointed out that in the case of a random distribution, modifications such as those adopted in the present analysis, could quite easily mask the fact that on any given day during the period of oviposition plants in a certain state of development are tending on the whole to receive more eggs than the other plants.

Marshall's data consisted of the numbers of eggs on each of several hundred maize plants, recorded on two separate occasions with an interval of a week. The eggs take about four days to hatch, so that each count gives the sum of the eggs laid on a plant during a period of four days. In the whole experimental plot the total for the second count was more than 2.5 times as great as the total for the first count. In other words we might say that during the second period of four days the plot was on the whole 2.5 times as attractive as it was during the first four days. But what the present data do not show is the day to day development of this attractiveness. Again, for the calculation of the theoretical curve to correspond with each of the egg counts it was necessary to assume that the plants were divided into certain arbitrary numerical groups. For example, in the first distribution, $\frac{1}{7}$ of the plants were assumed to receive an average number of eggs equal to the average of the whole population, but a further $\frac{1}{7}$ of them received an average of nearly three times as many eggs, while the average for the remainder was only about 0.1 times that of the population. But even if, in fact, the plants could be classified in these three grades of average attractiveness, which would be expressed in terms of average development, this would be only a crude representation of what was really happening in the field.

The processes of development are continuous. Any one plant passes through an infinite number of developmental stages, and at any one instant the plants in a field exhibit, within the limits defined by the most advanced and the least advanced plant, a very large number of stages of this same development. In time, and to a certain extent, in space also, the natural phenomena show continuous variation, but the mathematical method employed has dealt with this feature to a very limited extent. The plants were allocated

to groups, the averages for each of the groups being widely different from each other. Yet the plants within each group were treated mathematically as if they were all attractive to exactly the same degree. The building up of the egg distribution curve from three or four small curves was an attempt to get nearer to a picture of what one imagined was a possibility in the field. To return to the analogy of the marbles dropping into boxes, the idea of the compartments being of different sizes represents the plants in different stages of attractiveness. We could, if necessary, deal with as many sizes of compartments as we liked in calculating the random distributions. This would correspond with the range in attractiveness of the plants on any one day. They also vary in relation to one another with the passing of time. Because the data in the present problem represent the oviposition for two separate periods of four days, the relation between the plants on the two occasions could be represented only by a displacement in the fitting together of the two sets of theoretical curves—*vide* the text data referring to Figs. 3 and 4. A more exact mathematical treatment of a problem of this kind would need, as a basis, a concept like the one in which marbles were supposed to drop at random into the compartments, but certain sets of these compartments were replaced by new ones from time to time. In the account given earlier in the paper, a step by step substitution of the 100 compartments in lots of 10 was described. But the substitution could be imagined to take place in a less disjointed manner and at a varying rate, so that the total number of compartments in use is not necessarily constant. In this way the compartments that were actually receiving marbles at any instant could correspond to the maize plants, which were, for example, the most attractive during any given day, but the calculation of a random distribution among all those compartments would present some difficulty.

The Greenwood–Yule Distribution

A mathematical investigation of a similar problem embodying this concept of continuous variation was made by Greenwood and Yule (1). The practical application of their work was the analysis of data derived from certain records including those of the numbers of accidents occurring in groups of factory workers and the frequency distribution of those accidents among the individuals of the groups. It was readily shown that accidents did not happen altogether at random among the members of the several groups of workers which is no less than we would expect from a brief consideration of the great differences that exist between one man and the next. The theoretical random distribution as represented by the simple Poisson series therefore did not fit the accident frequency distribution. This led to an examination of modifications of the random distribution theory including what is described as the "infinitely compound Poisson distribution". The authors say— "We now suppose that the population at risk consists of persons (or other variates) the liabilities of whom to accident vary, the frequencies being assigned by the ordinates of $f(\gamma)$) where γ is a variable parameter." It was first of all assumed that $f(\gamma)$ is a normal function, but this hypothesis was discarded

for two reasons, namely, that the resulting compound Poisson series was rather unmanageable and that "values of γ may be supposed to range from zero far in the positive direction so that $f(\gamma)$ should be skew. A choice of skew curves is arbitrary." The form actually adopted is the curve corresponding to the skew binomial. It is a Pearson Type III curve and has the equation

$$y = y_0 e^{-c\gamma}\gamma^{r-1}.$$

When it is integrated in conjunction with the terms of the series representing simple random distribution, viz.

$$e^{-\gamma}\left(1 + \gamma + \frac{\gamma^2}{\underline{|2}} + \frac{\gamma^3}{\underline{|3}} + \ldots\ldots\right)$$

the following series results

$$\left(\frac{c}{c+1}\right)^r\left(1 + \frac{r}{c+1} + \frac{r(r+1)}{\underline{|2}\,(c+1)^2} + \frac{r\,(r+1)(r+2)}{\underline{|3}\,(c+1)^3} + \ldots\ldots\right).$$

The values of the mean and the second moment are respectively $\frac{r}{c}$ and $\frac{r(c+1)}{c^2}$.

The distribution is therefore easily fitted from the mean and second moment of the observations.

Before discussing the applicability of the hypothesis of the Greenwood–Yule series to the problem of the distribution of *Heliothis* eggs we shall compare the observations with the theoretical distributions. In the three cases the constants 'r' and 'c' are found to have the following values.

	1st Count	2nd Count	Sum of counts
c	0.50298	0.21873	0.21834
r	1.24392	1.25840	1.79613

The respective distributions are shown in comparison with the data in Table V, where the numbers underlined indicate that the frequencies in the remaining classes have been summed. They are illustrated in Figs. 5, 6, and 7. In the case of the first egg count the Greenwood–Yule distribution in Fig. 5 is obviously not such a good fit for the data as the theoretical distribution in Fig. 1. The irregularities of the second count prevent a really good fit and there is apparently little to choose between the two distributions that have been selected to represent it. We might attempt to lessen the effect of the irregularities in question by making assumptions as to how they may have arisen. But that would involve further assumptions of what theoretically the nature of the distribution ought to be. Such hypotheses could not be justified, for the aim of the investigation is to discover what the distribution actually is. To state what it should be, would be to reason in a circle. Whatever we may suspect about the accuracy of the observations, we are not, at this stage, entitled to challenge them on theoretical grounds, and we must accept Marshall's statement that they were made, under his supervision, with all due care, and with adequate checking. One point of interest in the comparison between the Fig. 2 distribution and the Greenwood–Yule distribution

TABLE V

No. of eggs per plant	1st Count		2nd Count		Sum of two counts	
	Data	Greenwood–Yule distribution	Data	Greenwood–Yule distribution	Data	Greenwood–Yule distribution
0	206	200.4	101	90.0	45	35.7
1	143	165.8	72	92.9	54	52.6
2	128	123.8	89	86.1	50	60.3
3	107	89.1	84	76.8	54	62.6
4	71	62.9	66	67.1	61	61.7
5	36	43.9	40	57.9	68	58.7
6	32	30.4	56	49.5	53	54.5
7	17	20.9	43	42.1	50	49.9
8	14	14.3	49	35.7	39	45.0
9	7	9.8	28	30.1	46	40.2
10	7	6.7	39	25.4	31	35.6
11	2	4.5	13	21.3	26	31.4
12	3	3.1	17	17.9	26	27.4
13	3	2.1	12	14.9	36	23.9
14	1	1.4	14	12.5	27	20.7
15	1	1.0	12	10.4	15	17.9
16	4	1.9	10	8.7	16	15.4
17			11	7.2	8	13.3
18			7	6.0	13	11.4
19			1	5.0	15	9.7
20			3	4.2	9	8.3
21			1	3.5	7	7.1
22			2	2.9	4	6.0
23			2	2.4	5	5.1
24			1	2.0	3	4.3
25			0	1.6	4	3.7
26			1	1.4	8	3.1
27			1	1.1	0	2.6
28			0	1.0	0	2.2
29			7	4.4	1	1.9
30					0	1.6
31					1	1.3
32					1	1.1
33					1	0.9
34					5	4.9

in Fig. 6, is that the former seems to be a better representation of the middle section of data curve, while the latter is distinctly superior for the section to the right of the curve. In fact in each of these cases the Greenwood–Yule distribution gives a better representation of the numbers of plants with high numbers of eggs. The reason for this will be found in an examination of the basic hypotheses of the Greenwood–Yule distribution. It assumes that the plants vary in their attractiveness to the ovipositing moths, and this variation is expressed by an equation which states that a diminishing fraction of the plants may be increasingly attractive with a limit at infinity. The relation between the fraction of plants and the degree of attractiveness is variable in that the fraction may diminish more or less rapidly, although the limit of attractiveness is theoretically never less than infinitely great. Because the mathematical constants of the Greenwood–Yule distribution are derived

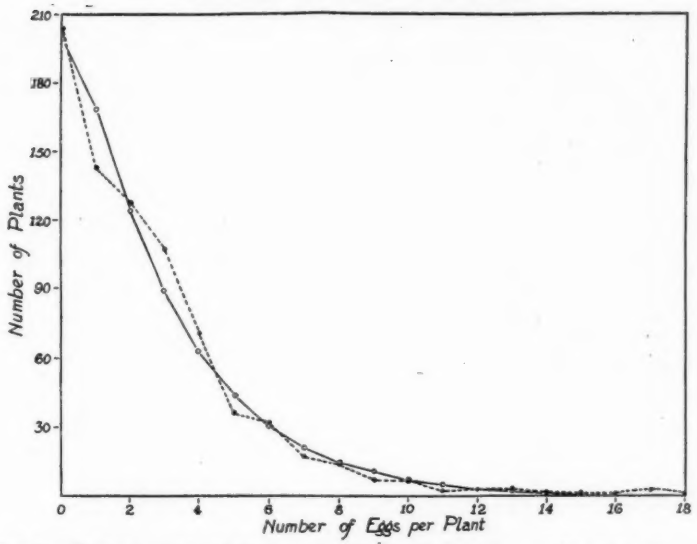

FIG. 5. *First egg count and Greenwood–Yule distribution;* ●----● *= data,* ○——○ *= Greenwood–Yule distribution.*

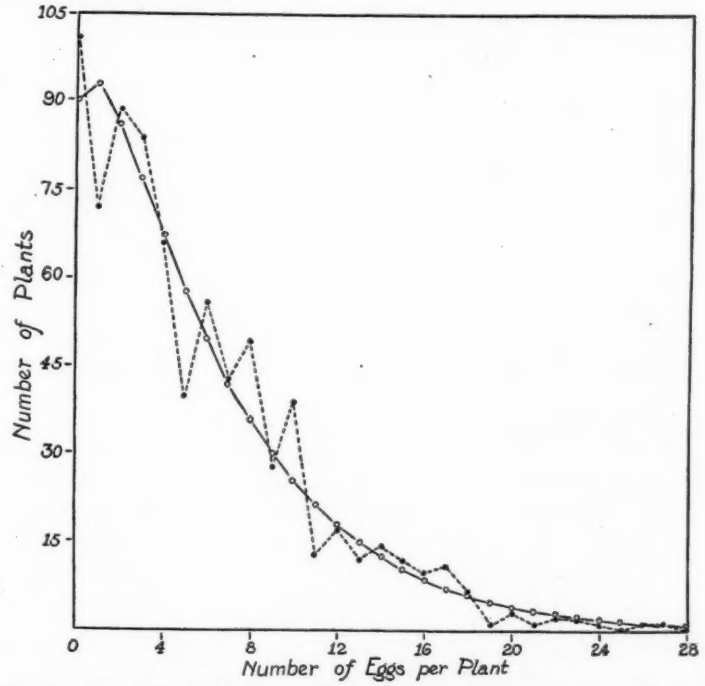

FIG. 6. *Second egg count and Greenwood–Yule distribution;* ●----● *= data,* ○——○ *= Greenwood–Yule distribution.*

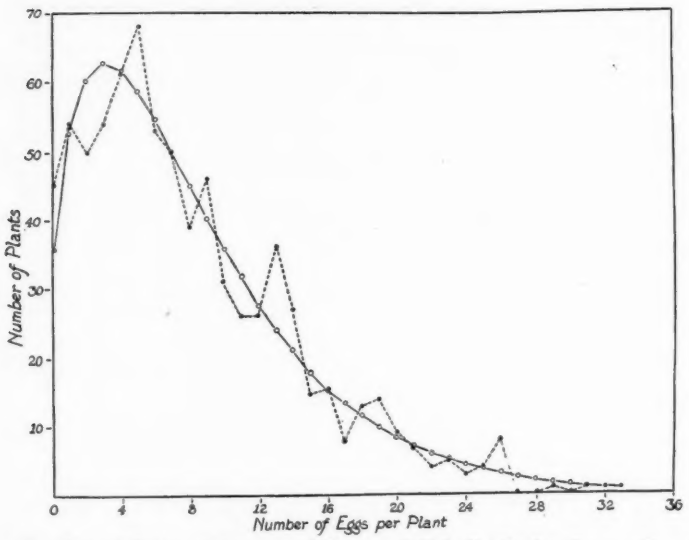

FIG. 7. *Sum of the two egg counts and Greenwood–Yule distribution;* ●- - - -● *= data,* ○——○ *= Greenwood–Yule distribution.*

from the data with which it is to be compared, the curve tends to adjust itself to the needs of the data. On the other hand, with the author's method of compounding an arbitrary series of simple random distributions into a single curve, it was desirable to keep the number of these distributions as low as possible in order to avoid too much clumsy arithmetic. The very attractive plants are therefore treated as one group, and although the resulting curve is asymptotic to the horizontal axis, the ordinate value tends too rapidly to zero.

The Greenwood–Yule frequency for the sum of the two counts fits the data fairly well for the numbers of eggs greater than five per plant, but it reaches a maximum in the wrong place. In this respect it is, however, better than the theoretical distribution of Fig. 3. At this stage, to recall the difference between the construction of Fig. 3 and Fig. 4, may help to explain what seems to be the inadequacy of the Greenwood–Yule curve in this particular case. In Fig. 3 the plants are assumed to have retained the same *relative* attractiveness during the oviposition periods prior to each count. In Fig. 4 the plants most attractive in the first count are assumed at the second count to be on the decline in respect of their choice by moths, and to have been superseded by others. The Greenwood–Yule distribution for both counts together was derived directly from the actual distribution for the sum. The constants about which it is built up are therefore those most suited to illustrate the data. What it cannot allow for is the essential difference between the sum and each of its two component parts. Within each count there is a continuity of events, but between the counts there is a distinct gap. The interval between the two occasions of counting the eggs was a week and it is recorded that

Heliothis eggs hatch in three or four days. Therefore there was no record of the eggs laid during the three days succeeding the first count. If the author's hypotheses are correct, their distribution would presumably have indicated an intermediate stage in the relative attractiveness of the maize plants.

The important point is that the Greenwood–Yule distribution assumes that there is variation among the plants in the likelihood of their receiving each of the individual eggs. It was mentioned above that the original problem to be investigated was the occurrence of accidents among factory workers, the hypothesis being that the persons concerned varied in their tendency to meet with accidents. For accidents to happen the passage of time is required, particularly if some individuals are to be involved in more than one of the accidents. But the individuals themselves are not considered to be affected by the passing of time. Those who at the outset are not likely to meet with accidents remain like that. Those who are prone to accidents do not become more careful as the result of mishaps. The susceptibility of each person is determined by his inherent physical and mental make up. Most people know from experience that there is a certain amount of truth in the saying that a burnt child dreads the fire, but a general theory based on a parallel idea did not explain the distribution of accidents (1). The mathematical formula that did in fact give adequate representations of the accident distributions incorporated the theory that the individual persons differed in their liability to accidents, but did not learn through experiencing them to avoid them. This same formula does, as we have shown, give a fairly good representation of the distribution of *Heliothis* eggs among the plants in a plot of maize. It is reasonable to suppose that the plants vary in their attractiveness to the female moths; i.e., in their liability to receiving eggs. So far, the basic hypothesis of the formula is acceptable, but it is not quite adequate to explain all the facts in the present problem.

Discussion

The object of this paper is to explain the observed distribution of the eggs of the moth, *Heliothis armigera*, on maize, which is one of the insect's most important host plants.

The observed distribution, when plotted by graphical methods, presents certain irregularities in its details, but its general trend can be represented by a smooth curve, the appearance of which suggests that the phenomena that it represents conform to some mathematical law.

It is possible that the irregularities themselves are significant and due to errors in sampling of some particular kind; but this suggestion is derived from cases that have little or no relation to those discussed in the paper and the known facts do not support it.

The observed distribution of the eggs of *Heliothis* on the plants represents the end result of a multitude of complex and continuous interactions between the insect, the host plant, and the rest of the environment. These interactions cannot be brought under direct observation and it is impossible to

retrace the history of the events in the fields studied. We cannot, therefore, subject our explanation to an exact observational test.

Nevertheless, there are two criteria by which we can judge its value. We are dealing, in the first place, with a series of numbers, arranged in a certain manner. We have formed the impression that it is an *ordered series*; i.e., one in which the values follow one another according to a *mathematical law*. When we have discovered the mathematical law, we have one of the most important parts of the explanation. In the second place, we have to deal with actual living organisms of certain specific kinds, behaving in certain characteristic ways that have been observed and described. Our explanation must take these into account, or, at least, it must agree with such observed facts as are relevant to the process that we are discussing.

It is clear that *both* requirements must be met if our explanation is to be considered adequate. To find the mathematical law of the distribution (supposing, which may not be the case, that its law can be defined with absolute certainty) is not enough. We could not *explain* the form of a liquid drop completely by proving that it had the equation of a sphere; we would have to show, in addition, how the known properties of liquid matter *actually engendered this form*. Similarly, in the present case, we must not only find the mathematical law according to which the eggs of *Heliothis* are distributed; we must also show the connection between this law and the observed characteristics of the moth and the developing maize plant. An explanation that is based on physical and biological phenomena of other categories and that has no demonstrable connection with moths and maize plants, cannot be accepted as adequate, even though its mathematical results agree well enough with the data.

We can go even farther than this. Because, owing to inevitable errors in sampling and to the irregularities in natural phenomena, trends that are truly fundamental may be distorted and masked, it is usually difficult to be quite sure just what mathematical law a given process really follows. The tendency to choose a curve that fits reasonably well simply because it is derived from a known mathematical expression, may lead us badly astray. It may be, that the true mathematical law, based on the biological facts, is less elegant or easily expressed and will not reveal itself unless the facts are taken as a starting point. In short, our explanation must be a *biologico-mathematical explanation* and since we are in the domain of natural history, we are justified in insisting on the outstanding importance of its biological element.

In the region where the data were collected *Heliothis* breeds continuously for a considerable period, and, owing to the overlapping of the generations there is, during a good part of this time, a large floating population of moths ready to oviposit.

Parsons and Ullyett proved definitely that the attraction of the plants to the moths is a quality that changes with the passage of time. They demonstrated (1) that the appearance of moth eggs in a particular crop of maize is due to the attraction of moths from neighbouring regions, and (2) that when the

oviposition rate diminishes, it means that the surviving moths have left for other, more attractive areas. Thus, the rate of oviposition at any time depends on the number of moths present, that is to say, on the degree of attractiveness of the crop as compared with other neighbouring crops.

As the plants in a field begin to mature, the moths in the neighbourhood are attracted in increasing numbers to the field. The rate of oviposition rises rapidly and the number of eggs attains a maximum by the time all the male flowers or tassels have appeared. At this time only 42% of the tassels are fully extended and only 50% of the silks have appeared, but 70% of the eggs have been laid. When, later, all the tassels are completely developed, the rate of oviposition is almost negligible and by the time that 100% of the cobs have full silking, oviposition has ceased in that field.

Marshall's data showed that the eggs are certainly not distributed either uniformly or at random among the plants. The obvious inference is, that some plants are more likely to receive eggs than others. We may take it that the movements of the moths are random movements in the sense that they do not search the plot of maize in an orderly or systematic manner: do not, for example, go up one row of plants and down the next. We may also call them random, meaning that the movement of a given moth from its resting place may be to *any* point of the compass. But this is merely because the most attractive plant at the moment may be at any point of the compass, since there is no definite relation between the spatial arrangement of the plants and the degree of attractiveness. Though the movements of the moths would seem random to a casual observer and may really be random, with respect to compass points, they are not random with respect to the character of the individual host plants. However, since the attractiveness of the whole crop changes, increasing to a maximum and then diminishing, according to the proportion of plants that are in the various stages of development, it is not unreasonable to assume that with the passing of time there is a change in the relative as well as in the absolute attractiveness of the individual plants. Surveys made at different times could not, therefore, be expected to give similar spatial distributions of eggs.

Now the type of explanation of egg distribution that we are attempting to produce requires the description of the biological situation in a form susceptible of mathematical representation. Only quantitative elements can be included in the mathematical representation. Nevertheless, the pattern or arrangement of these elements must be that of the real situation: in this case, a pattern characteristic of the moth population ovipositing in the maize fields. But one can imagine a series of such patterns all based on events in the maize field, but differing greatly in complexity. Thus the problem of the oviposition of *Heliothis* could be called the problem of distributing x units among y units. It would then be mathematically simple, but would probably not lead to a correct solution because certain important features of the real problem had been omitted. On the other hand, an effort to include everything that really affects the distribution of *Heliothis* eggs might result in a mathematical repre-

sentation so complex as to be unworkable. Only a biologist acquainted with natural conditions and the behaviour of the organisms considered can say how many elements must be incorporated in the mathematical outline and even the biologist's judgment is subject to revision as the knowledge of the problem progresses and the results of preliminary attempts at explanation become available.

Let us now review briefly the main possible explanations of egg distribution.

A glance at the data shows that the eggs are not distributed uniformly among the maize plants. For example, all plants do not bear eggs; the plants bearing these have not always the same number nor do they recur in the same places in the plot. The simple idea that the problem is that of x things distributed at random among y things (simple Poisson distribution) was originally tested by Marshall, but as has been already said, the curve developed from this theory differs markedly from the curve of the data. One explanation of this might be that the maize plants differ in their receptiveness to eggs, or, to put the thing in another way, in their attractiveness to the moths. If the maize plot is large enough, and the conditions in it sufficiently varied, there might be so many intermediates between the least and the most attractive plants, at any given moment, that the variation would be practically continuous. The compound Poisson series, derived by Greenwood and Yule, may be applied to the *Heliothis* problem if we assume that the variation of the maize plants in attractiveness, at any given moment, is continuous and expressed by a skew curve—Pearson, Type III.

There are, however, a number of serious objections to this solution of the problem. In the first place it was developed from the study of an entirely different problem—the distribution of industrial accidents—which has, biologically, nothing whatever in common with the distribution of *Heliothis* eggs in maize plants, although it is possible to construct a vague general definition covering both cases. In the second place, it is not certain that in the relatively small plot studied by Marshall, the maize plants at any given moment formed a continuous series with respect to their attractiveness. It is possible, and even probable that they formed a number of fairly distinct groups, differing rather markedly in attractiveness and connected by relatively few intermediates, because there is often, in a maize crop, a rather marked difference between the dominant and subdominant plants owing to the shade cast by the former. If a graph of the various types were plotted one might, by joining the ordinates, get a rough approximation to the skew curve; but this would not prove that the variation was continuous. In the third place, though the characteristics of the maize plant probably do not form a continuous series in *space*, as is implied in the Greenwood–Yule system, they do almost certainly change in a perfectly continuous manner in *time*, because they wax and wane with the growth of the plant, which is a practically continuous phenomenon. This continuity in time is not taken into account in the Greenwood–Yule system. The system involves some rather difficult mathematics. The constants that define the curve are, as we have seen, derived

from the data so that it is the closest fit that could be obtained for a curve of this class. Nevertheless, it is, in fact, only a moderate fit for the data, so that the defects in its biological foundations are not compensated by the perfection of the numerical results.

The hypothesis developed by the present writer has been erected on the basis of what we actually know about the relation of *Heliothis* during the period of oviposition to the developing maize plant. The object of the work has been, in the first place, to produce this curve from a statement that is biologically accurate. It is not claimed, of course, that the hypothesis is immune to criticism. The representation of the variation in time in the maize plant as a discontinuous process is admittedly diagrammatic and the variation in space at a given moment may be much less definite and discontinuous than is implied in the mathematical treatment adopted. Relatively simple mathematics have been used, because the differences among the maize plants have been considered to be discontinuous. The curves actually obtained, as we have seen, fit the data somewhat better than the Greenwood–Yule curves; and in so far as the combined count is concerned, this is apparently due in part to the fact that the basic Greenwood–Yule hypothesis does not take the facts into account.

It seems therefore justifiable to say that the hypothesis of egg distribution put forward in this paper is satisfactory as a first approximation. In spite of the simplicity of the mathematical treatment employed, the results obtained fit the data rather better than those given by the Greenwood–Yule hypothesis. Further investigations, based on more extensive collections of field data, may enable us to construct a better theory; but no theory, no matter how elegant its mathematical development, nor how exact the numerical 'fit' it gives, can be considered acceptable to biologists unless it is constructed on a foundation of known biological facts.

Acknowledgments

The writer is indebted to the Committee of the Carnegie Trust for the Universities of Scotland for the Fellowship during the tenure of which this investigation was made. Thanks are due also to Dr. W. R. Thompson, F.R.S., for his interest in the problem, and to Mr. G. Udny Yule, C.B.E., F.R.S., for very helpful advice.

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